UNIVERSITY OF ALBERTA

THE TIMING OF CALCIUM INTAKE AND ITS EFFECT ON THE CALCIUM METABOLISM OF BROILER BREEDER AND LAYING HENS

by

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ABSTRACT

Poor eggshell quality is responsible for lost income in both the hatching egg and table egg industries. Two studies were performed in order to determine dietary means to increase eggshell quality, and consequently bone mineral content. In the first study, the effects of the timing of increasing dietary Ca levels to young broiler breeder hens were investigated. Dietary Ca levels were increased at various ages and the effects examined under thermoneutral and heat-stress environments. When Ca levels were increased 2 wk before, or 6 wk after photostimulation, eggshell quality was diminished. Increased eggshell quality often came at the expense of bone quality in the heat-stress environment indicating that while the timing of increasing dietary Ca level is important in a thermoneutral environment, it was even more so under heat-stress conditions. The second study investigated the use of midnight feeding to encourage feed consumption during the dark period (when most eggshell formation occurs) and subsequently, to increase eggshell quality and bone mineral content of Leghorn hens. The hens were divided into 2 experiments; long-term (31.5 wk) and short-term (4.5 wk) midnight feeding. There were no differences in overall feed consumption, egg traits, or bone mineral content between the midnight fed and daytime fed treatments in either study. The results of both studies, along with past research, suggest that the timing of Ca intake plays a critical role in Ca metabolism in both broiler breeder and laying hens.

DEDICATION

This thesis is dedicated to my family. My Mom, Dad, and Kyle have offered me more support than I could have asked for and all that I needed. This was a much longer road than I had anticipated, but I learned more than could be taught in any classroom or by performing any research. Thank you for standing by me and encouraging me in the darkest times.

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LIST OF ABBREVIATIONS

BW	body weight
CP	crude protein
CT	calcitonin
ME	metabolizable energy (a calculated value expressed in Kcal/kg)
PTH	parathyroid hormone
QCT	quantitative computed tomography

1.0 INTRODUCTION

In the egg-laying domestic hen, there is a constant Ca demand for the maintenance of skeletal integrity and eggshell formation. Once the hen begins egg production, she is presented with the challenges of supplying Ca for metabolic processes daily, and over the length of the egg production cycle. In the layer industry, it is estimated that poor shell quality results in 7 % of all eggs with laid are not able to be sold for human consumption (Roland *et al.*, 1996). This number is impossible to estimate in the broiler breeder industry.

1.1 EGG FORMATION

A description of egg formation was given by Johnson (1986). The oviduct of the laying hen can be divided into four sections; the infundibulum, magnum, isthmus, and shell gland. The funnel-like infundibulum "catches" the follicle once it has been released and sends it on its way down the oviduct. In the magnum, layers of albumin (made of protein synthesized with amino acids from the blood) are laid down around the yolk. This step takes approximately 4 hours. In the isthmus, two shell membranes (an inner and an outer membrane) are deposited around the albumin. When these membranes are first laid down, they are quite tight around the albumin; however, by the time the egg enters the shell gland, they have stretched and become quite loose. The first five hours that the egg is in the shell gland, it undergoes a process called "plumping" which is the entry of water and salts through the membranes. This process draws the membranes tight again and is essential for eggshell formation and deposition to begin. The shell is attached to the outer membrane by mammillary knobs which are formed between the isthmus and the shell gland. Calcite crystals begin to form around the mammillary knobs, by the binding of Ca ions to the sulfonic acid groups present in the mammillary cores. The main part of the shell is called the

palisade layer, and is composed of columns of tightly packed calcite crystals. The spaces between the crystals are the pores of the shell, which run from the surface of the shell to the membranes. These pores are used by the embryo to take in oxygen and excrete carbon dioxide during incubation. The deposition of the shell usually takes 15 to 16 hours. The final stage in the formation of the eggshell is the addition of a waxy layer called the cuticle, which is deposited just before oviposition (Johnson, 2000).

The domestic hen is not a seasonal breeder, but lays continuously throughout the year. A sequence (defined as the number of consecutive days an egg is produced) can continue for months at a time. The average eggshell contains 2.3 g of Ca, which is equivalent to approximately 10 % of the Ca content in the entire skeleton (Etches, 1987). As a result of this constant challenge to maintain Ca equilibrium, the hen has developed efficient systems for Ca management and turnover.

Ca metabolism is dependent on several factors including intake, absorption, retention, and excretion. Ca intake can be influenced by the time of day the hen has access to feed or the age at which the bird begins to receive a high Ca diet to support egg production (Dacke, 1979). Absorption of Ca is influenced by the stage of the ovulatory cycle (Hertelendy and Taylor, 1961). More Ca is absorbed from the gut during periods of eggshell formation than during non-laying periods (Hurwitz and Bar, 1965). Ca retention can be assessed by measuring bone characteristics such as ash content, which is an indicator of Ca content, and Ca excretion is generally assessed by measuring eggshell quality which is affected by the amount of Ca being deposited on the eggshell. Variables within each of these factors can affect the overall Ca balance in an egg-laying bird, thereby affecting the health and profitability of the flock. Attention must be given to feeding programs, lighting programs, and other

environmental factors, such as temperature, in order to optimize the Ca output in the form of marketable eggs.

1.2 Ca INTAKE

Obviously, intake is the first limiting factor in Ca utilization. Deficiency of Ca in the diet, whether short-term (for example, overnight during eggshell formation) or long-term (the entire production cycle), results in increased dependence on bone Ca and inability to sustain egg output. Etches (1987) determined that Ca intake is under the influence of several factors. There is a circadian rhythm influencing feed intake, and by extension, Ca intake. When Ca is present in the feed and the hen is unable to separate it from the other feed ingredients, Ca intake will proceed at a constant rate throughout the day in *ad libitum* fed hens (Mongin and Sauveur, 1974). If given the choice, a hen will selectively consume Ca at the end of the photoperiod in an effort to supply as much Ca to the digestive system as possible in anticipation of the dark period (Mongin and Sauveur, 1974). The stage of the ovulatory cycle also affects Ca intake. If given free-choice access, a hen will consume 25 % more feed on days when eggshell formation is occurring than on days when it does not (Johnson, 2000). When readily available, most of the Ca required for eggshell formation is derived directly from the digestive tract. However, if there is a limited amount of feed readily available in the gut, or the Ca concentration of the feed is lower than 3.6 %, Ca from other sources will be utilized. Even though dietary Ca may be less than required, ovarian follicles can still be ovulated (Herteledy and Taylor, 1961); however, without a viable eggshell, an egg cannot be sold regardless of its purpose (hatching or human consumption).

While Leghorns, which produce table eggs for human consumption, and broiler breeders, which produce hatching eggs (hatched for broiler or meat

production) both produce eggs, the rearing, management, and genetic makeup of the two are quite different, resulting in differences in the way Ca is provided. Intensive breeding has resulted in differences between Leghorns and broilers in intestinal villi, affecting the nutrient uptake by the gut (Yamauchi and Isshuki, 1991). The same is assumed to be true between Leghorns and broiler breeders. Broiler breeders are thus expected to have villi with increased surface area to facilitate nutrient absorption required for the rapid growth and heavier body weight associated with those breeds. The intensive breeding for weight gain in the broiler has resulted in the need to restrict feed intake of the breeder in order to keep egg production high (Robinson *et al.*, 1993). The feed restriction also has the unwelcome effect of restricting the amount of Ca the hen consumes, and more importantly, the time at which she consumes it. Intensive breeding for egg numbers has had the opposite effect on Leghorns. Appetite has been reduced and as a result, the challenge with Leghorns is to coax them to consume more feed. The layer producer relies on egg numbers for profitability; the breeder producer needs both high number of eggs and hatchability of those eggs. Both types of producer can increase profitability by increasing eggshell quality.

In order to control body weight, broiler breeders are commonly weighed on a daily or weekly basis, and based on the body weight recommended by the primary breeder, feed allocation is determined. Any deviation from the recommended body weight can result in poor egg production, so the effort is made to adhere to the recommendations. As a result, broiler breeders are fed once per day, usually in the morning and have short feed clean-up times. It is common in commercial broiler breeder flocks to use "5 + 2" feeding to control body weight before the hens begin to lay. The weekly feed allocation is divided into 5 equal allotments, and fed on 5 days out of a week. The "skip" days, in which no feed is given are not consecutive.

Typically, the schedule involves feed for 3 days, a "skip" day, feed for 2 days and another "skip" day. Hudson *et al.*, (1999) reported increases in bone surface area covered by osteoclasts (the cells that break down bone) in broiler breeder hens fed on a skip-a-day system (similar to a "5 + 2" system) as compared to those fed a limited amount daily. This indicates that if hens are forming medullary bone when on a "5 + 2" schedule (which is possible just before the hens begin to lay) there may be a greater reliance on the breakdown of cortical bone, than in those hens fed less on a daily system. This is a problem unique to the broiler breeder industry due to the necessity of feed restriction. In contrast, Leghorn hens are generally given *ad libitum* access to feed with feeders running when the lights are on. Neither feeding system takes into account that most eggshell formation occurs at night, and feed is generally inaccessible to the birds.

Typically, both Leghorns and broiler breeders are fed versions of a starter, grower, and layer ration in order to meet the nutritional demands of growth and production. The introduction of each diet must be carefully planned as to meet the demands of the majority of the flock, however the most important transition appears to be that between the low Ca grower and high Ca layer diets. Several studies have been conducted examining the effect of dietary calcium in pre-lay (grower) diets on subsequent reproductive performance (Hurwitz 1964, Hurwtiz and Bar 1969, Anderson 1967, de Andrade *et al.* 1977, Tanor *et al.* 1984, and Clunies *et al.* 1992) however most research has been done in layer-type chickens rather than broiler breeders. Differences in egg production level as well as feeding patterns (morning versus all day) between Leghorns and broiler breeders require additional research to investigate the effect of pre-lay diet changes in broiler breeder hens.

Hurwitz and Bar (1971) reported that in Leghorns, there is no danger in increasing Ca levels 1-2 months before laying. However, the phosphorous level and the P:Ca ratio in the pre-lay diet does play a role in egg production with high phosphorous reducing production when accompanying a high Ca diet. The researchers also concluded that increased dietary Ca helped increase bone mineralization and shell quality, however neither pre-lay Ca or P levels affected bird response to Ca deficient diets fed one month after the onset of egg production.

1.3 Ca ABSORPTION

Once consumed, Ca is not released from the crop at a constant rate throughout a 24-hour period (Roland *et al.*, 1972) and may not be readily available to the hen during the dark period when the majority of shell formation is proceeding. Unfortunately, increasing the amount of dietary Ca does not increase the amount available in the gut for the future. By supplying more Ca, a larger amount of Ca is released from the gut immediately instead of the hen retaining the Ca and releasing more over a longer time. For example in Leghorn hens fed a Ca supplement, 43% of the Ca was absorbed within 4 hours after feeding and only 15 % was absorbed between 4 and 8 hours after (Roland *et al.*, 1973). The same authors theorized that a hen laying an egg at 6:00 pm has twice as many hours of light during the shell-forming period as a hen laying an egg at 6:00 am. Therefore a greater proportion of shell calcification is carried out when the hen has access to feed and more Ca in the digestive tract to utilize. Increased supply of Ca directly from feed rather than medullary bone results in higher eggshell quality. Roland *et al.* (1973) found that the specific gravity is higher in eggs laid in the afternoon than in the morning due to the availability of feed in the digestive tract during shell calcification. Broiler breeder

hens, fed the entire daily feed allocation in the morning, are therefore at a disadvantage.

Intestinal Absorption

When dietary Ca is consumed at or above required levels, most Ca will be absorbed by passive diffusion. Primary Ca absorption occurs in the duodenum and upper jejunum (Hurwtiz, *et al.*, 1973) and is largely regulated by Vitamin D (Castillo *et al.*, 1977). Once Ca is absorbed, it moves to the vascular system for transport. The uptake of Ca from the gut is affected by many factors. Feed (and therefore Ca) consumption and the rate at which Ca is absorbed by the intestine both affect the rate at which Ca enters the vascular system. The intestine plays an active role in controlling Ca absorption rather than acting solely as an entry point. Both Ca absorption and its entry into the vascular system are affected by time of day, stage of the ovulatory sequence, and the source of Ca in the feed, as well as the stage of eggshell formation. A large diurnal increase in Ca absorption was observed in laying hens during the hours of eggshell formation (Hurwitz and Bar 1965). Once calcification was complete, Ca absorption returned to normal (Hurwitz *et al.*, 1973).

Blood Plasma Concentration

Blood plasma concentrations of Ca are maintained between 20-25 mg/100 mL (Winget and Smith, 1958). The majority of eggshell calcification usually occurs during the dark period (Etches, 1987), when the lights are off and the hen is not consuming feed. During this time, the shell gland of the hen will extract 2.0-2.5 g of Ca from the blood and medullary bone and transfer it to the eggshell without storing it in the interim (Eastin and Spaziani, 1978). This depletion of Ca from the blood occurs at a rate of 100 – 200 mg/h, which if not replenished, would completely drain plasma Ca reserves in 15 minutes (Etches, 1987). Since eggshell calcification lasts

approximately 15 hours (Eastin and Spaziani, 1978), an alternate source of Ca must be available to replenish blood plasma levels and allow the transfer of Ca to the shell gland to continue without interruption. Under normal circumstances, the mechanism in place to ensure Ca supply includes the medullary bone.

1.4 Ca RETENTION

Medullary Bone in Egg-laying Hens

The bone biology of the laying hen changes considerably at sexual maturity. In response to estrogen, pullets develop a unique type of labile bone prior to the onset of egg production called medullary bone (Bloom *et al.*, 1941). Medullary bone is a type of non-structural bone located in the marrow cavity of the long bones and is formed approximately 14-16 days before the onset of egg production (Hurwitz, 1964). Skeletal weight increases 20 % during this period (Riddell, 1992) and as a result, the Ca requirements of the hen increase substantially. If her Ca requirements are not met during this time, the hen will not develop sufficient medullary bone to sustain production (Hurwitz, 1964). While the most rapid build-up occurs during this period, medullary bone may continue to form throughout the production cycle if sufficient dietary Ca is maintained (Hurwitz and Bar, 1966).

Medullary bone is responsible for providing Ca to the hen when there is no feed present in the digestive tract, and eggshell formation is occurring (Taylor and Moore, 1954), for example during the dark period. The turnover rate of medullary bone is almost double that of cortical bone (Hurwitz and Bar, 1965), demonstrating its ability to be both deposited and mobilized quickly. Bloom *et al.*, (1958) observed that the period of shell calcification was marked by an initial rise in both osteoblasts (bone forming cells) and osteoclasts (cells that break down bone cells) in the medullary bone, followed by a sharp decrease in osteoblast numbers and an increase in

osteoclasts. Candlish (1971) found high levels of acid phosphatase, (which reflects osteoclastic activity) during periods of eggshell formation, and alkaline phosphatase (which reflects osteoblastic activity) when eggshell formation is not occurring. A net loss of Ca from the medullary bone occurs when eggshell formation proceeds during the dark period, whereas a net gain of medullary bone proceeds during the day, when the hen has access to feed, regardless of the stage of eggshell formation (Etches, 1987). Candlish (1971) reported that during shell calcification, the shell gland competes with the medullary bone for blood Ca. In fact, 13 to 15 h after the egg entered the shell gland, the shell gland is more successful than medullary bone in competing for Ca from the blood. If the only feed of the day is supplied to the hen during this time, the medullary bone will not be replenished with Ca. This underscores the importance of providing feed to laying hens over the course of the day, and possibly overnight.

Effects of Hen Age

Hurwitz and Bar (1969) reported differences in the way young and old laying hens utilized bone Ca. The state of the bone Ca reserves directly affects the ability of the hen to lay eggs while consuming a Ca deficient diet and, that the state of these reserves are directly related to the age of the hen; older hens have more. This suggests that young hens (near peak production) are more susceptible to reduced or interrupted dietary Ca both on a daily or long-term basis. When hens first come into production, shell quality is good, even though little medullary bone may be present. Whitehead (2003) hypothesized that while dietary sources of Ca are preferentially used, the Ca for the eggshells laid early in production can come from mobilized structural bone and not medullary bone. In fact, at peak production, the laying hen cannot absorb enough Ca from the diet to meet her requirement for shell formation

and must mobilize bone reserves to meet the demand of eggshell formation. When there is insufficient Ca for eggshell formation coming from the digestive tract and medullary bone, structural bone can be mobilized. Additionally, structural bone may be attacked by osteoclasts if it is not covered by a layer of medullary bone (Whitehead, 2003). Whereas medullary bone provides Ca in the absence of dietary Ca, cortical bone appears to provide Ca during periods of chronic Ca deficiency. Hurwitz and Bar (1969) reported that the cortical bone in young hens is more labile than that of old hens, and the ability of the hen to utilize cortical bone decreases with age. Since cortical bone is responsible for strength, supplying young hens with sufficient Ca, so that they are not forced to utilize structural bone, is of utmost importance to limit the number of hens experiencing osteoporosis or bone breakage. Farmer and Roland (1986) observed that the greater the dependency on skeletal Ca (medullary and cortical bone), the less Ca is deposited on the eggshell. Whitehead (2003) determined that bone mass formation in broiler breeder hens, prior to lay, is of the utmost importance because it will be used by the hens throughout the laying cycle. The same study indicated that little cortical bone formation occurs once pullets begin to lay, increasing the importance of pre-lay Ca nutrition in broiler breeder pullets. If little medullary bone is formed when the hen is young, she will have a smaller supply to utilize later in life. McCoy and Reilly (1996) determined as hens aged, structural bone (trabecular and cortical bone) reached peak mass at first ovulation and continues to decrease in mass throughout lay, whereas medullary bone is initially formed 10 to 14 days before first egg (Hurwitz, 1964).

Effects of Dietary Ca

Clunies *et al.* (1992) suggests that the Ca level in the pullet diet directly affects the amount of medullary bone reserves and hens with larger reserves show an

increased shell output. However, the relationship between dietary Ca and medullary bone reserves is not linear. Clunies *et al.* (1992) reported hens fed Ca at 2.5% or 4.5% showed less medullary bone formation than those fed a diet with 3.5% Ca. Cheng and Coon (1990) reported that medullary bone levels were more sensitive to changes in dietary Ca levels than cortical bone.

Bone Strength

Leghorns are typically reared in cages and broiler breeders are almost always housed on the floor of the barn. The differences in housing have significant effects on bone strength and Ca metabolism for the two breeds. Hens kept in cages have been shown to have bones with lower breaking strength than those reared and kept in floor pens (Fleming *et al.*, 1994). Body weight is also a predictor of bone strength (Fleming *et al.*, 1994). Broiler breeder hens typically exhibit greater body weights than Leghorns, and therefore tend to have stronger bones.

Of the numerous studies performed on bone mineral characteristics of egg-laying birds, the emphasis has been placed on Leghorn hens, rather than broiler breeders. Osteoporosis in caged hens was first described by Couch (1955) who observed laying hens in a condition of paralysis or death. Hens with osteoporosis have brittle bones, caused by the erosion of cortical bone due to sustained egg production. While cortical bone can be utilized with relative ease in periods of Ca deficiency, it cannot be built up as easily again during the production cycle, even when Ca levels are sufficient, especially as hens age. When hens go out of lay, during a molt for example, estrogen levels decline, and structural bone can be re-formed (Whitehead and Fleming, 2000). The progressive loss of structural bone throughout the production cycle can result in brittle bones and cause high incidences of broken bones near the end of production and even into spent hen processing. The attrition of

structural bone can cause the collapse of vertebrae, causing the paralysis often associated with osteoporosis or cage-layer fatigue (Whitehead and Wilson, 1992). Genetics are believed to be a large cause of the problem. Laying hens are bred for low body weight, eat little, and maintain high Ca outputs for prolonged time periods (Whitehead and Wilson, 1992). In addition to genetics, there are nutritional and environmental factors. Nutrition and housing also play key roles in determining the susceptibility of hens to osteoporosis and caged layer fatigue. Recently, a syndrome similar to caged layer fatigue has been reported in broiler breeder hens in addition to Leghorns.

Measuring Bone Strength

Several methods of determining and predicting bone strength in poultry have been studied. Although cortical, and to a certain extent, trabecular bone are responsible for bone strength, medullary bone does play a role. Fleming *et al.* (1998) found that the humeral breaking strength of bones is directly related to the amount of medullary bone present in the bone; the higher the medullary bone, the greater the breaking strength. Although medullary bone has little intrinsic strength, it appeared that the interconnected lattice-like structure of the bone contributed to the overall strength. In the same study, the radiographic density of the bones was measured. It was found that the *in vivo* radiographic density of bones at 25 wk of age could predict which birds would have the strongest humeri, and therefore most medullary bone at the end of the production cycle. Fleming *et al.* (1994) determined that breaking strength and radiographic density were closely related. Bone breaking force (Kg) is sensitive to dietary Ca level (Cheng and Coon, 1990), with dietary Ca levels from 3.0 % to 4.5 % producing bones with the highest bone breaking force. While bone breaking strength is a useful tool to determine the Ca status of the hen, the condition

of the bone prior to the measurement can jeopardize accurate results. Lott *et al.* (1980) measured fresh, frozen, and oven dried bones and determined that oven drying bones decreased bone breaking strength by 50%. Only minor differences were observed between fresh and frozen bones.

Quantitative computed tomography (QCT) is a relatively new tool in determining bone density, and by extension, bone strength. First used in human patients, QCT is a non-invasive method of determining bone density in live birds (Korver et al., 2003). McCoy and Reilly (1996) found a significant ($p<0.001$) linear relationship between bone density using QCT and breaking strength in Leghorn hens. Other methods of measuring bone strength *in vivo* include single or dual energy X-ray absorptiometry (SXA or DXA), digitized fluoroscopy (DF), and ultrasound (Fleming *et al.*, 2000). The advantage possessed by these methods is the ability to scan the bones of live hens to make a determination of flock health and welfare.

1.5 Ca EXCRETION

Shell Deposition

Blood plasma supplies all of the material required to form the calcite crystals that make up the palisade layer of the eggshell. The shell gland is highly vascularized to allow for the delivery of raw materials used to form the eggshell. Two kinds of Ca are found in the blood of laying hens during egg production; protein bound (also referred to as non-diffusible), and ionic (diffusible). The protein phosphovitin is synthesized in the liver and has a high affinity for Ca. Phosphovitin is the characteristic protein of the egg yolk and carries Ca to be incorporated into the yolk. Ionic Ca is the form of Ca used for eggshell formation. The two forms of Ca in the blood appear to be in equilibrium with each other. Once blood passes through the shell gland, there is a drop in the concentrations of both protein-bound and ionic Ca

(Winget and Smith, 1958). While the shell gland takes the ionic Ca, the protein-bound Ca is too large to diffuse, so it is believed that the protein and Ca dissociate with one another to maintain the equilibrium (Johnson, 2000).

The eggshell itself contains about 60% carbonate. A complex equilibrium exists among H_2O, CO_2, H_2CO_3, HCO_3^-, CO_3^{2-}, H^+ and OH^-. The formation and precipitation of the carbonate radical involves changes in the concentrations of all seven of the previously listed species. CO_2 is hydrated in the mucosa of the shell gland to form HCO_3^-, a step catalyzed by the enzyme carbonic anhydrase. When birds are heat stressed, panting decreases the amount of CO_2 present and therefore eggshell quality suffers. $CaCO_3$ is precipitated onto the forming shell as a result of its affinity to the organic matrix on the surface of the shell.

1.6 GENERAL Ca METABOLISM

The regulation of Ca concentration in body fluids is achieved through the action of a complex feedback-control system that includes several subsystems and regulating hormones. The mechanism regulating Ca homeostasis is an endocrine feedback loop consisting of parathyroid hormone (PTH), Vitamin D (1,25 (OH_2) cholecalciferol), and calcitonin (CT) which interact with the intestine, kidney, and bone, the target tissues (Lobach, 1996). The amount of available Ca varies throughout the day as a result of periods of feeding and fasting. The hen has homeostatic systems in place to prevent vast fluctuations in circulation Ca levels. Plasma Ca levels are normally in balance with gut, bone, and kidney Ca levels, and any change in input or output that is not counterbalanced results in Ca imbalance. Hormonal control of gut, bone, and kidney processes is used to respond to such imbalances.

Hormones

Ca metabolism is regulated by both steroid and peptide hormones. The peptide hormones, PTH and CT have short half-life and respond to plasma Ca concentrations within minutes, while steroid hormones take longer (Johnson, 2000). Bone is the immediate target for the action of both PTH and CT.

PTH

Changes in blood Ca concentration are sensed by cell-surface receptors on the parathyroid gland (Lobach, 1996). In response to low Ca concentration in the blood, PTH is secreted from the chief cells of the parathyroid gland. PTH preserves constant blood ionized Ca levels by increasing renal Ca absorption, mobilizing Ca from the skeleton, and increasing absorption of Ca from the gut by stimulating the production of Vitamin D (Lobach, 1996). Once released, the primary targets of PTH are the bone and kidney cells. In bone, PTH receptors are present on osteoblast surfaces (Hurwitz, 1989) and appear to increase the permeability of bone-cell membranes. Ca diffuses by passive transport from the bone cell to the blood serum, thereby increasing plasma Ca levels. Although no PTH receptor has been discovered on osteoclast cells, high doses of PTH will stimulate bone resorption resulting in higher serum Ca levels (Lobach, 1996). In the kidney, PTH reduces the resorption of Ca, sodium, and P in the proximal renal tubule. However, it increases absorption of Ca in the distal portion of the renal tubule resulting in the net retention of Ca (Lobach, 1996).

CT

Calcitonin has two functions: to correct hypercalcemia, and to protect the skeleton from excessive resorption (Brown, 1991). CT is released from the ultimobrachial gland (positioned near the parathyroid glands) in response to Ca concentrations of greater than 1.5 mmol/L (Brown, 1991). Hypercalcemia usually

arises immediately before or after ovulation, when Ca has been mobilized from the digestive system and bone reserves, but is not quite being absorbed and utilized by the shell gland (Lobach, 1996). CT inhibits the action of osteoclasts thereby limiting bone resorption.

Vitamin D

Vitamin D is a steroid hormone provided in the diet, or synthesized in the liver in response to increased PTH levels. There is both direct and indirect response of the Vitamin D endocrine mechanisms by low blood plasma Ca levels (hypocalcemia). Vitamin D is hydrolyzed by the liver to form 25-(OH)-D_3. A second hydroxylation to 1,25-$(OH)_2$-D_3 is completed in the kidney in response to high levels of PTH or low levels of Ca or P. In a hypocalcemic state, Vitamin D increased both the passive and active transport of Ca from the duodenum into the blood (Tanaka *et al.*, 1976). 1,25-$(OH)_2$-D_3 induces the formation of Ca-binding protein to increase the rate of Ca absorption from the small intestine (Lobach, 1996). Parathyroid gland cells contain receptors that, when occupied by the 1,25-$(OH)_2$-D_3 molecule, inhibit PTH secretion (Tanaka *et al.*, 1976).

1.7 QUESTIONS THAT REMAIN

In both Leghorn and broiler breeder barns, profitability is reduced by poor eggshell quality; however, the underlying causes are very different. In broiler breeders, the emphasis is on egg production and fertility, and to be fertile, the flock's weight must be controlled through feed restriction. Problems in shell quality can arise during peak egg production, when the hen may not be able to absorb enough Ca from the feed or medullary bone stores, to maintain high levels of eggshell production. Two outcomes of insufficient Ca are poor eggshell quality or poor bone quality, both

resulting in lost production. Feed restriction in and of itself is a stress to the hen, but when combined with other stresses such as excessive heat or the ability of the hen to reproduce is greatly reduced. This problem is exacerbated by such factors as poor flock uniformity which makes it difficult to judge the appropriate time to change from low Ca grower to a high Ca layer diet. Supplying sufficient Ca at the right time of the laying cycle is critical as studies have shown that Ca given too early may be detrimental to medullary bone formation and can lead to problems later in life. There is an obvious drawback to providing increased Ca to hens after the majority of the flock has begun laying. Determining the best time to increase Ca to a young breeding flock before production starts, especially when there are external stressors, is critical to the financial viability of the breeder operation.

In Leghorns, poor eggshell quality usually appears at peak production, and as flocks age. The genetic makeup of the birds is such that they are not as motivated to eat as a feed-restricted bird bred for weight gain and, therefore, may not consume sufficient Ca. Leghorns have been bred for efficiency; to produce the high number of eggs on the least amount of feed, effectively breeding against appetite. Bred for high egg production, by the end of the cycle, the hens rely heavily on medullary bone Ca stores and dietary Ca. Breeding the hens for increased egg output did not increase appetite, therefore, another solution must be found in order to increase Ca intake of Leghorns to sustain the high Ca output. One such method may be midnight feeding. The theory behind midnight feeding is to supply Ca to laying hens during the dark period when most shell formation occurs.

Objectives

The objectives of the first study were to determine the effect of timing of increased dietary Ca (relative to photostimulation) to broiler breeder females at two

environmental temperatures (Chapter 2). The objectives of the second study were to determine the effects of long-term and short-term midnight feeding on the eggshell and bone characteristics of Leghorn hens (Chapter 3).

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2.0 BROILER BREEDER EGG PRODUCTION AND QUALITY ARE AFFECTED BY TIMING OF INCREASED DIETARY CA RELATIVE TO PHOTOSTIMULATION

2.1 ABSTRACT

Ross 508 broiler breeder pullets (n=400) were reared in floor pens in a light-tight facility with feed allocation based on breeder-recommended body weight targets. At 17 wk, 250 pullets within 15 % of the mean weight were randomly assigned to individual cages in one of two environmentally-controlled environments: 18°C (Experiment 1, n = 125) or 28°C (Experiment 2, n = 125). The pullets were photostimulated at 20 wk of age. At 2 wk intervals from 18 to 26 wk, one group of hens (n=25 per environment) was switched from a 0.88 % Ca grower diet to a 2.96 % Ca breeder diet. Dietary treatment names were based on the time (in wk) relative to photostimulation that the diet was changed from the grower to the breeder diet (PS-2, PS, PS+2, PS+4, and PS+6, respectively). The hens were killed at 31 wk of age and one leg from each hen was taken for bone mineral analysis. The birds in both experiments maintained target body weights as described by the primary breeder, however the hens in Experiment 2 did not consume the allotted feed for the last two weeks. Dietary treatment did not affect feed consumption or body weight. Overall % hen day production was not affected by dietary treatment in either experiment. In Experiment 1, hens in the PS+4 and PS+6 treatments produced larger eggs and hens in the PS+4 treatment produced eggs with heavier shells. Shell weight as a percentage of egg weight was significantly lower from the PS+6 hens. The PS+4 treatment had the highest bone Ca as a percentage of ash weight indicating that four wk after photostimulation was the optimal time to increase bone Ca reserves in that

environment. In Experiment 2, the PS+6 treatment had the highest egg weight while the PS and PS+2 treatments had the highest shell weights. Percent shell was the lowest in the PS+6 and PS+4 treatments overall, although it was only significantly different until 27.4 wk of age. Ca as a percentage of bone weight was lowest in the PS+2 and PS+6 treatments and Ca as a percentage of ash was lowest in the PS+2 treatment. Overall the results suggest that inappropriate timing of increasing dietary Ca either before or too long after photostimulation decreased eggshell quality and bone characteristics. Environmental temperature exacerbated these effects, therefore the change from a low Ca grower to a high Ca breeder diet must be made with greater precision in hot climates.

2.2 INTRODUCTION

Calcium metabolism of Leghorn and broiler breeder hens is similar, yet vast differences in management and feeding practices exist between the two. Whereas considerable research has been done on the Ca requirements of the Leghorn, little has been done with the feed-restricted broiler breeder hen. The main emphasis of broiler breeder research has been on controlling body weight to keep egg production high and few studies have examined the importance of pre-lay and pre-peak dietary Ca on Ca metabolism during egg production.

Calcium Resorption from the Medullary Bone

The medullary bone is a highly specialized type of bone that is used by the hen as a labile source of Ca. Medullary bone can be either built up or broken down rapidly in response to Ca supply and needs of the hen. When insufficient Ca is absorbed from the digestive tract, Ca from the medullary bone is mobilized into the blood (Simkiss, 1967). At peak egg production, the hen may not be able to absorb enough Ca from the diet to meet her requirement for shell formation and must therefore rely on medullary bone to provide the Ca to sustain egg production.

Hurwitz (1964) determined that medullary bone in Leghorn hens is formed 14 to 16 d before the first oviposition, under the influence of reproductive hormones. The skeletal weight of Leghorns increases 20 % in the two wk before the first oviposition and as a result, the Ca requirement of the hen increases substantially during this time (Riddell, 1992). This period is likely the optimal time to change from a low-Ca grower diet to a high-Ca breeder diet, as birds will be physiologically capable of depositing increased Ca into the medullary bone.

Clunies *et al.* (1992) reported that 40 wk old laying hens fed a 4.5 % Ca diet showed less medullary bone formation than those fed a 3.5 % Ca diet. In that study, more Ca was supplied by the diet than was needed for eggshell formation, but the excess was excreted rather than stored as a defence against future Ca deficits. Although Clunies *et al.* (1992) used older Leghorn hens it is possible that feeding a high Ca diet to pullets prior to medullary bone formation may also be ineffective in protecting against future dietary Ca insufficiencies.

The optimum time to change the diet from a low Ca grower diet to the high Ca broiler breeder diet may not be solely dependant on the age of a flock. Rather, characteristics such as hypothalamic maturation (initiated by light stimuli), body weight and time relative to photostimulation are better predictors of sexual maturation, and therefore, when medullary bone formation will occur (Robinson *et al.*, 1993). Hypothalamic maturation is considered to be the state when the bird is physiologically capable of responding to photostimulation by initiating the onset of sexual maturity, as mediated by the hypothalamus.

Effect of Pre-lay Feeding Programs

Several studies have examined the effect of dietary calcium levels in pre-lay diets on subsequent reproductive performance of laying hens (Hurwitz, 1964; Hurwitz and Bar, 1969; Anderson, 1967; de Andrade *et al.*, 1977; Tanor *et al.*, 1984; Clunies *et al.*, 1992). Hurwitz and Bar (1971) reported that there was no detrimental effect of increasing Ca from a pre-lay diet (0.5 %) to a layer diet (3.5 %) 1 to 2 months before the onset of production in laying hens. Increasing the Ca level at that time appeared to increase bone mineralization and shell quality at the onset of egg production. In that study, pre-lay Ca

level had no effect on the rate at which shell quality declined when the hens were fed a Ca-deficient diet one month after egg production began. Those results suggest that there is a physiological limit to the amount of medullary Ca that can be stored; simply increasing dietary Ca may not protect against future situations in which demand is greater than dietary supply.

Environmental Temperature

Although several studies have been conducted on the effects of heat stress on egg production, most have been performed under conditions of short-term heat stress or cyclic variations due to the lack of precise environmental controls (de Andrade *et al.*, 1977). Also, the little work done in this area has been on the interaction of heat stress and diet on laying hens rather than feed-restricted broiler breeder hens. Tanor *et al.* (1984) reported decreased feed consumption and body weight due to short-term heat stress and de Andrade *et al.* (1976) reported similar results under constant, long-term heat stress. de Andrade (1977) reported that heat stress decreased egg production and blood pCO_2 in hens. Since CO_2 is a precursor to calcium carbonate (the structural component of eggshell), eggshell quality may be compromised by heat stress-induced panting and subsequent increase in CO_2 exhalation. de Andrade *et al.* (1977) and Mueller (1959) indicated that decreases in shell quality were in response to heat and not a result of decreased feed consumption or Ca intake.

The objective of the present experiments was to study the effect of timing of increased dietary Ca (relative to photostimulation and first ovipositon) at two environmental temperatures on body weight, feed consumption, egg production, egg shell quality, blood chemistry, carcass traits and bone mineral content. The first hypothesis

tested was that increasing Ca either before photostimulation or near peak production, would reduce egg production, egg shell quality, and bone mineral content of broiler breeders. The second hypothesis tested was that birds in the high temperature (28 C) environment in Experiment 2 would more affected by the extremes in dietary treatments in terms of egg production, egg shell quality and bone mineral content as compared to those in the thermoneutral environment (18 C) in Experiment 1.

2.3 MATERIALS AND METHODS

Stocks and Housing

Four hundred Ross 508 broiler breeder pullets (Aviagen North America, Huntsville, AL, USA, 35805) were reared in floor pens in a light-tight facility with 24 h of light per day for the first 3 d and with 8 h of light per d to 20 wk of age. The pullets received a commercial-type starter diet containing 1.00 % Ca, 18.21 % CP, and 2,783 kcal ME/kg (Table 2-1) from hatch to 3 wk of age. From 3 to 17 wk of age all pullets were fed a commercial-type grower ration containing 0.88 % Ca, 15.04 % CP, and 2,711 kcal ME/kg. Birds were individually weighed on a weekly basis; feed allocation was based on breeder-recommended body weight targets.

Experimental Design

At 17 weeks of age, the pullets were individually weighed and 250 birds within $\pm$15% of the mean weight were selected for the experiment. The mean weight was the same as the target weight recommended by the breeder guide (Ross 508 Breeder Management Guide). The birds were fed following a 5 + 2 feeding regime (fed five days per wk and not fed Sunday and Wednesday) until 17 wk of age to keep the body weight of the flock uniform, when they were transferred into the laying barns. The birds were

randomly divided into one of two environments: 18°C (Experiment 1, n = 125) or 28°C (Experiment 2, n = 125) where they were individually housed and fed in laying cages. The two experiments were run concurrently. At 20 wk, the photoperiod was increased from 8 h to 11 h per d. The photoperiod was increased an additional 1 h per d on a weekly basis until it reached 16 h per d when the birds were 24 wk of age. Within each experiment, the hens were randomly assigned to one of five dietary treatments. Dietary treatment names were based on the number of wk relative to photostimulation that the diet was changed from the grower to the breeder ration, ranging from 2 wk prior to photostimulation to 6 wk after photostimulation. Beginning at 18 wk, two wk before photostimulation (PS-2 treatment), 25 hens per experiment were changed from the 0.88 % Ca grower ration to a commercial-type breeder ration containing 2.96 % Ca, 15.8 % CP, and 2,619 kcal ME/kg (Table 2-1). Subsequent groups of hens (n = 25 per group) within each experiment were switched to the high Ca breeder ration at 20, 22, 24, and 26 wk of age (PS, PS+2, PS+4, PS+6 treatments, respectively). Individual body weight data of all birds was recorded once per week; feed allocation was based on average body weights in each of the experiments. The entire feed allocation was provided to the hens once per day and daily feed intake was recorded. Once per week, a blood sample was taken from two hens per diet (n = 10 per week per experiment) for blood chemistry analysis (pO_2, pCO_2, Na and pH). All eggs with normal eggshells were weighed, and two eggs were collected each week from each hen (Monday and Friday) for measurement of specific gravity using the flotation method (Nordstrom and Ousterhout, 1982). The range of saline solutions used was 1.062 to 1.094 in increments of 0.002. Weight of the shell without the membranes was measured by first rinsing the shells in warm water and gently

massaging the inside of the shell to remove the membrane; the membrane-free shells were then weighed. At 31 weeks of age the birds were killed by cervical dislocation and the body weight was recorded. Carcasses from the birds were dissected to determine *Pectoralis* major (*P.* major), *Pectoralis* minor (*P.* minor), liver, heart, oviduct, and stroma (the ovary without the large yellow follicles, that is follicles greater than 10 mm in diameter) weights. The left leg was removed from each carcass and frozen for subsequent bone ash and Ca analysis.

Bone Mineral Analysis

To determine the degree of Ca retention and bone quality, a bone mineral analysis was performed on the hens at 31 wk of age. Each entire leg was thawed and placed in a drying oven for 6 h at 100°C to loosen the soft tissue to allow for easier separation from the bone; the toes, foot, and all other remaining soft tissues were removed from the shank after cooking. The bone was placed into an aluminum cup and weighed to determine the wet bone weight. The bone and aluminum cup were placed into a drying oven at 100°C overnight to remove all moisture. After removal from the oven, the bones and aluminum dishes were placed in a dessicator to cool. Once cool, the bones were weighed again to determine a dry bone weight. The bones were transferred into weighed ceramic crucibles and placed into an ashing oven at 600°C overnight. To cool, the crucibles were placed in a dessicator. The bones were allowed to return to room temperature, weighed, and ash weight was determined. The ashed bone was crushed into a fine powder, mixed and 200 mg of the ash was weighed into a 75 mL beaker. Fifteen mL of 1:1 HCl:distilled, de-ionized water and 10 drops of concentrated nitric acid were added to the ash and stirred until dissolved. The volume was diluted to 50 mL with a 0.5 % $LaCl_3$ solution in water.

A 100 μL aliquot of the sample solution was transferred to a volumetric flask and further diluted to 50 mL with $LaCl_3$. Five standard solutions (0, 1, 2, 3, and 4 ppm) were prepared by serial dilution of a 1,000 ppm Ca reference solution (Fisher Scientific, Nepean, ON, Canada K2E 7L6). Samples and standards were read at the same time with a Perkin-Elmer Model 4000 Atomic Absorption Spectrophotometer (Norwalk, CT, 06856) at a wavelength of 422.7 nm to determine Ca content.

Statistical Analysis

Two experiments were performed, with diet as the main effect. The birds were individually housed; the bird was the experimental unit. Data were analysed as a one-way ANOVA using the General Linear Models (GLM) procedures of SAS® (SAS Institute, 1999). All statements of significance were assessed at $P \leq 0.05$. Seven hens in Experiment 2 died during the course of the experiment (not from Ca-related illness) and were removed from the data set. The SEM (standard error of the mean) values reported were based on the treatment or interaction group with the fewest birds.

2.4 RESULTS

Body Weight and Feed Consumption

The birds in both experiments were managed to maintain target body weights as described by the primary breeder. On average, the hens in Experiment 2, consumed 440 g less feed in the last 2 wk of the experiment. In Experiment 1, the total feed intake per bird was 2238 g in the last two wk and in Experiment 2, the total feed intake for the same period was 1798 g. The body weight at the end of the trial of the hens in Experiment 1 was 3.448 kg, and 3.334 kg in Experiment 2. No significant differences in body weight or feed consumption were observed among dietary treatments at any age.

Egg Production

Overall % hen day egg production (the number of hens laying an egg as a percentage of the number of live hens in the barn) was not affected by dietary treatment (Table 2-2). Hens in Experiment 2 reached 50 % production sooner, but had a lower peak egg production than hens in Experiment 1. The hens in Experiment 2 reached peak weekly production (75.7 %) at 26 wk of age while the hens in Experiment 1 reached peak production (87.4 %) at 28 wk of age. Total egg production over the course of the studies was similar between Experiments 1 and 2 and among dietary treatments within each experiment (data not shown).

Egg Weight

In Experiment 1, the average egg weight over the course of the experiment was significantly greater in the PS+4 and PS+6 treatments (Table 2-3). In Experiment 2, the PS+6 treatment had the highest egg weights overall and the PS-2 treatment had the lowest (Table 2-3). The overall average egg weight produced by the hens in Experiment 1 was 56.30 g (± 4.95g) while the overall average egg weight in Experiment 2 was 54.19 g (± 5.56g).

Shell Weight

In Experiment 1, over the course of the experiment (Table 2-4) eggs from the PS-2, PS and PS+6 hens had lower shell weights than the PS+4 treatment. The highest shell weights in Experiment 2 were from hens in the PS and PS+2 treatment groups (Table 2-4). The average shell weight across dietary treatments was 5.01 g (± 0.57 g) in Experiment 1, and 4.80 g (± 0.59 g) in Experiment 2.

Shell Weight as a % of Egg Weight

The overall percent shell in Experiment 1 was 8.91 % (± 0.84 %) as compared to 8.88 % (± 0.90 %) in Experiment 2 (Table 2-5). In the thermoneutral environment of Experiment 1, there were no significant differences among dietary treatments from 29 wk until the end of the trial, however in Experiment 2, there were no differences from 27.4 wk until the end (Table 2-5). The PS+6 treatment resulted in the lowest shell percentage in both trials. Egg specific gravity (data not shown) followed the same pattern.

Bone Mineral Analysis

In Experiment 1, the PS+4 treatment resulted in significantly higher Ca as a percent of ash weight than all other treatments except PS-2 (Table 2-6). In Experiment 2, the PS+2 treatment group had the lowest Ca as a percentage of ash weight of all the treatments (Table 2-6). Ca as a percent of bone was highest in the PS+4 and PS-2 treatments and lowest in the PS+6 and PS+2 treatments in Experiment 2.

2.5 DISCUSSION

Body Weight and Feed Consumption

The feed consumption of the hens in elevated temperature conditions of Experiment 2 declined at the end of the trial, as did body weight. When hens are feed restricted to maintain breeder-recommended target body weights, their feed intake is below the level of voluntarily consumption. It is possible that, earlier in Experiment 2, any reductions in voluntary feed intake were masked by the level of feed restriction imposed. As the end of the experiment approached (29 and 30 wk of age), the voluntary feed intake of the hens in Experiment 2 dropped below the amount that was being fed and therefore, reductions in feed intake were observed. The experiments were run

concurrently, with birds from the same initial population, therefore the differences in feed intake and body weight are likely attributable to the differences in temperature. Previous work on heat stress in egg laying birds has involved non-feed restricted table egg strains of hens. Vo *et al.* (1978) found the feed intake of Leghorn hens raised at 35 C was half of that of hens kept at 21 C. Loss of appetite has also been reported in hens subjected to heat stress, accompanied by decreases in body weight (de Andrade *et al.* 1976). One theory is that hens reduce feed intake during heat stress because of the thermic effect of feeding. Physical activity is also decreased during heat stress with the exception of panting, thus further lowering the energy requirements of the hen.

Egg Production

In both experiments, the PS+2 treatment peaked at the highest level; 90.3 % in Experiment 1, 81.1 % in Experiment 2; the difference was not significant in either case. Across dietary treatments, the hens in Experiment 1 reached peak production (87.4 %) at 28 wk of age while the hens in Experiment 2 reached peak production (75.7 %) at 26 wk of age. Hen day percent production was measured using all eggs produced, including eggs with poor eggshell quality and soft-shelled eggs to demonstrate whether or not differences in production were a result of changes in ovulation rate or shell quality. In a commercial breeder operation, eggs with very poor shell quality are likely to break before or during collection, and may therefore reflect artificially low egg production. The individual caging system used in the present experiments allowed the enumeration of ovipositions, rather than the production of eggs with sufficient shell quality to arrive at a collection point. The average total number of eggs produced per hen to 31 wk of age was 36 in Experiment 1 with the average number of ovulations being 36. In Experiment 2,

the average number of eggs produced per hen to 31 wk of age was 34 with 34 ovulations, demonstrating that differences in shell quality did not affect percent production. Stockland and Blaylock (1974) found that laying hens reared at 29.4 C laid fewer eggs from 20 to 46 wk of age than those reared at ambient room temperature.

Egg Traits

The relationship between dietary treatment and hen age may be best explained when examined in terms of Ca intake (feed consumption) and Ca output (production level and eggshell deposition). When placed on the breeder diet, the PS-2 birds in both experiments had a high Ca input from the diet, and a low Ca output because this group was not yet producing a large number of eggs. At that time, it is unlikely that medullary bone formation had begun in the majority of those hens, and the excess Ca was most likely excreted. Early in production, the hens in the PS-2 treatment were producing eggs with a low percent shell even though the egg size was also low (below the 52g lower limit for hatching eggs in Canada; F. E. Robinson, personal communication) until 26 wk. The number of eggs produced per hen was not affected by dietary treatment, suggesting that limited Ca availability for eggshell formation resulted in hens in the PS-2 group laying smaller eggs, rather than reducing egg numbers in order to decrease the amount of Ca needed to maintain a functional eggshell. This is supported by Simkiss (1967) who observed smaller eggs produced by young hens under Ca restriction. The percent shell from the PS+2 and PS (Experiments 1 and 2) and PS-2, PS+4 birds (Experiment 1 only) was consistently higher than the other treatments. The variable results for the PS+4 treatment group indicate that there may be an interaction of environmental temperature with diet on eggshell quality. The PS+6 treatment consistently resulted in the poorest

shell quality in both experiments. The longer-term implications of poor Ca supply on egg numbers were not determined in the present study.

The PS-2 hens in the current trials probably excreted excess dietary Ca prior to onset of sexual maturity rather than storing it in bone reserves. When pre-lay diets of 0.9 % Ca and 3.5 % Ca were fed prior to maturity, Leeson *et al.* (1986) found no difference in daily Ca retention in the bone of Leghorn hens. In fact, more Ca was excreted by the hens fed the 3.5 % Ca diet, supporting the theory that giving Ca before maturity may be detrimental to the development of medullary bone reserves. At the other extreme, birds in the PS+6 treatment began egg production during a period of low Ca intake and produced eggs with the lowest shell weight, percent shell, and specific gravity at the beginning of egg production. The PS+6 hens were unable to meet the demand for Ca output as egg production increased and reached its peak near 28 wk or 26 wk (Experiments 1 and 2, respectively). After 28.4 wk or 26 wk (Experiment 1 and 2 respectively), there were no differences in shell weight as a percentage of egg weight (Table 2-5), suggesting that the PS+6 hens were able to return to a normal shell percentage when given sufficient dietary Ca. This supports the findings by Hurwitz and Bar (1966) that showed feeding a normal layer ration to Ca-depleted hens resulted in eggshell Ca returning to the same level as that of Ca-replete hens in 6 to 8 d. In Experiment 1, the eggshell showed this improvement 2.4 wk after the increase in dietary Ca while in Experiment 2, the eggshell improved immediately (at 26 wk). The eggs produced by the PS, PS+2, and PS+4 treatments had high shell weight, percent shell, and specific gravity throughout most of the experimental period. Thus, the timing of increasing dietary Ca to breeder hens relative to onset of sexual maturity has a major

impact on eggshell quality. Increasing dietary Ca too early (ie the PS-2) as well as too late (ie PS+6) are both potentially detrimental to egg production and shell quality.

The optimal timing of increased dietary Ca supply was also dependent upon the environmental temperature. In hens under no temperature stress (Experiment 1) the best eggshell traits in general were seen in the PS+4 treatment which had the among the highest egg weight, shell weight, and shell weight as a percentage of egg weight. Under conditions of heat stress (Experiment 2), the best results were observed in the PS and PS+2 treatments, which were consistently among the highest in egg weight, shell weight, and shell weight as a percentage of egg weight. Whereas the PS, PS+2, and PS+4 treatments resulted in equivalent levels of production and egg quality in Experiment 1, the results of Experiment 2 suggest that under conditions of heat stress, the change to a high Ca breeder diet has to be made with more precise timing. The best egg production and shell quality in Experiment 2 were in the PS and PS+2 treatments only. The PS+4 treatment resulted in different effects in each of the experiments in terms of peak egg production. The age at which the birds in this treatment group in the two experiments reached peak production may offer an explanation. The increase in dietary Ca to PS+4 hens in Experiment 2 occurred 2 wk before the hens reached peak production (at 26 wk of age), whereas the increase in Experiment 1 was given 3 wk before peak (at 27 wk of age), during a time when egg production in this group was very low. As a result, the hens in Experiment 2 hens may have had less of an opportunity to develop medullary bone reserves before peak production than the hens in Experiment 1. Lower medullary bone reserves would then have made the hens in Experiment 2 more susceptible to poorer eggshell quality, especially during peak production. This effect may be amplified

because broiler breeders, unlike Leghorns, are feed restricted and cannot consume extra feed to meet increased Ca demand for eggshell formation. Increasing the supply of dietary Ca prior to the expected initiation of medullary bone formation began decreased eggshell quality of broiler breeder hens.

Stockland and Blaylock (1974), Mueller (1961), and de Andrade (1976; 1977), all reported decreased egg size in heat-stressed Leghorn hens. Roland *et al.* (1996) found that differences in egg weight due to environmental temperature were due to differences in shell weight in Leghorn hens. As in the current experiment, Nordstrom (1973) showed that along with decreasing egg size, heat-stress also decreased shell weight as a percentage of egg weight in Leghorn hens. Huston and Carmon (1961) reported an overall decline in specific gravity as hens aged, and reported that this decline was more rapid in hens exposed to high environmental temperatures.

Bone Mineral and Calcium Content

The PS+4 treatment group in Experiment 1 had the highest bone Ca as a percentage of ash and eggshell quality. In Experiment 2, the PS+2 treatment had among the lowest Ca as a percentage of bone and of ash; the PS and PS+2 treatments demonstrated generally better egg traits. The PS+4 hens had the highest Ca as a percentage of bone and ash of any group in Experiment 2. This may be related to the significantly lower % shell deposited by the PS+4 hens, thus sparing bone Ca reserves. This experiment ended at 31 wk of age, future experiments might be designed to examine the long-term effects of increasing dietary Ca before or after expected medullar bone formation. Increasing the Ca level before expected initiation of medullary bone formation (i.e. the PS-2 treatment) improved bone Ca retention during production, but

came at the expense of egg size and eggshell quality. Thus, premature increases in dietary Ca supplementation appear to cause broiler breeder hens to preserve bone mass at the expense of eggshell quality. The effect on egg numbers seems to be less severe.

Hurwitz (1964) found that medullary bone is formed approximately 2 weeks before the first oviposition, and is accompanied by an increased demand for Ca. Therefore, the optimal time to introduce the high Ca breeder diet would be at or near initiation of medullary bone formation (i. e. the PS treatment in the current study). In the present study, the optimal time to increase dietary Ca for eggshell quality was at or soon after the initiation of expected medullary bone formation (i.e. the PS and PS+2 treatment groups). The treatments in which Ca was increased before or after this interval resulted in impaired eggshell quality. The PS+2 treatment resulted in several bone characteristics at 31 wk of age that were among the poorest of the dietary treatments. It must be stressed that these experiments ended at 31 wk of age and therefore did not address the implications of Ca supply over an entire laying cycle. In the long term, the ability of the PS+2 birds to sustain high levels of egg production and quality may be impaired by their reduced bone Ca status. The formation of high-quality eggshells appears to come at the expense of bone Ca stores. The treatments that resulted in increased bone mineral status (PS-2 and PS+4), tended to result in poorer eggshell traits. The timing of increased dietary Ca to breeder hens may affect the way in which the birds partition Ca between eggshell formation and maintaining bone mineral reserves. The PS+6 treatment resulted in poor eggshell quality and bone mineralization, indicating that breeder hens must be supplied increased dietary Ca sooner than 6 wk after photostimulation.

Table 2-1. Ingredient and calculated nutrient composition of broiler breeder diets

Ingredients	Diets		
	Starter	Grower	Breeder
	g/kg		
Wheat	442.3	344.2	337.6
Corn	141.4	164.4	143.1
Wheat shorts	75.0	150.0	12.9
Oats	50.0	125.0	100.0
Barley	50.0	100.0	150.0
Soybean meal, 48%	173.4	73.7	134.2
Limestone	16.5	17.2	76.8
Biofos[1]	15.8	8.6	10.6
Choline premix[2]	5.0	5.0	5.0
Broiler premix[3]	5.0	5.0	----
Layer premix[4]	----	----	5.0
Iodized Salt	3.8	3.3	2.8
L–lysine HCl[5]	0.3	1.6	0.3
DL–methionine[6]	1.4	1.3	1.7
Tallow	20.0	0.7	20.0
Monensin[7]	0.7	0.5	----
Nutrient Composition			
Crude protein, % (analyzed)	18.21	15.04	15.80
ME, kcal/kg	2,783	2,711	2,619
Linoleic acid, %	0.81	0.87	0.89
Fat, % (analyzed)	4.43	2.96	4.40
Fibre, % (analyzed)	3.74	4.81	2.40
Calcium, % (analyzed)	1.00	0.88	2.96
Available Phosphorous, %	0.47	0.32	0.34
Total Phosphorous, %	0.73	0.58	0.68
Potassium, %	0.70	0.59	0.66
Chloride, %	0.29	0.27	0.24
Sodium, % (analyzed)	0.19	0.17	0.14
Lysine, %	0.84	0.72	0.70
Methionine, %	0.41	0.34	0.40
Cystine, %	0.32	0.27	0.28

[1] Agri-Feed Products Limited. Winnipeg, MB. R2M 5P9

[2] Choline chloride provided at 100 mg per Kg of diet. BASF Toronto, ON. N1G 4T2.

[3] Supplied per Kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,500 IU; vitamin E 35 IU; menadione, 2 mg; D-pantothenic acid, 14 mg; riboflavin, 5 mg; folic acid, 0.8 mg; niacin, 65 mg; thiamine, 2 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.015 mg; biotin, 0.18 mg; iodine,

0.5 mg; iron, 100 mg; zinc, 80 mg; manganese, 70 mg; copper, 8.5 mg; selenium, 0.1 mg. BASF Toronto, ON. N1G 4T2.

[4] Supplied per Kg of diet: vitamin A, 12,000 IU; vitamin D_3, 3,000 IU; vitamin E 40 IU; menadione, 2 mg; D-pantothenic acid, 14 mg; riboflavin, 6.5 mg; folic acid, 1 mg; niacin, 40 mg; thiamine, 3.3 mg; pyridoxine, 6 mg; vitamin B_{12}, .02 mg; biotin, 0.1 mg; iodine, 0.5 mg; iron, 100 mg; zinc, 80 mg; manganese, 75 mg; copper, 15 mg; selenium, 0.1 mg. BASF Toronto, ON. N1G 4T2.

[5] Ajinomoto-Heartland, Inc. Chicago, IL. 60631-3421, USA.

[6] Degussa AG Canada Inc. Burlington ON. L7R 3Y6.

[7] Elanco Animal Health, Guelph, ON. N1G 4T2.

Table 2-2. Mean weekly egg production (hen day %) of Ross 508 hens housed at 18C (Experiment 1) or 28C (Experiment 2) and fed one of five dietary treatments

		Hen Age (wks)								
Main Effect	Trt[1]	22	23	24	25	26	27	28	29	30
		----------%----------								
Experiment 1[2]										
n[3]		28	133	406	476	707	756	791	742	679
Diet	PS-2	0.02	21.98	50.00	67.58	85.16	88.46	86.26	80.77	79.49
	PS	2.75	13.74	49.36	67.03	79.12	80.77	87.92	81.87	83.33
	PS+2	1.14	8.57	35.33	64.57	77.71	88.59	90.32	88.00	86.78
	PS+4	2.29	17.71	36.67	59.43	81.24	85.71	85.71	84.00	76.08
	PS+6	0.01	16.57	35.33	57.71	81.67	79.43	86.86	85.14	82.62
Overall mean		1.24	15.71	41.34	63.26	80.98	84.59	87.41	83.96	81.66
SEM		6.15	15.85	26.47	29.96	22.54	21.05	22.34	24.78	20.98
P value		NS	NS	NS	NS	NS	NS	NS	NS	NS
Experiment 2										
n		14	168	441	569	665	644	602	581	553
Diet	PS-2	0.01	18.86	57.33	74.29	73.71	72.00	69.14	65.34	62.67
	PS	0.57	18.86	56.67	70.78	77.97	78.29	78.23	74.89	72.66
	PS+2	0.57	16.00	49.23	70.65	81.14	72.57	69.72	72.11	65.94
	PS+4	2.29	16.98	40.89	60.57	73.65	73.14	68.97	66.89	57.44
	PS+6	1.65	23.08	50.94	61.54	71.98	70.32	64.29	59.34	55.49
Overall mean		1.01	18.76	51.01	67.57	75.69	73.26	70.07	67.71	62.84
SEM		5.61	17.93	21.33	26.77	23.80	21.35	24.69	20.21	19.76
P value		NS	NS	NS	NS	NS	NS	NS	NS	NS

[a, b, c] Within an experiment, means within a column (dietary treatment) with no common superscript differ significantly.

[1] Treatment name. PS = Photostimulation (20 wk), the number refers to the number of wk before (-) or after (+) photostimulation that the birds were switched from a low Ca grower diet to a high Ca breeder diet.
[2] In Experiment 1, birds were maintained at 18 C throughout the study, in Experiment 2, the birds were housed at 28 C throughout the trial.
[3] n = total number of eggs produced for each week of the study.
* < 0.05, ** < 0.01.

Table 2-3. Mean egg weight of Ross 508 hens housed at 18C (Experiment 1) or 28C (Experiment 2) and fed one of five dietary treatments

		Hen Age (wks)													
Main Effect	Trt[1]	25	25.4	26	26.4	27	27.4	28	28.4	29	29.4	30	30.4	31	Overall[2]
		----------	----------	----------	----------	----------	----------	(g)	----------	----------	----------	----------	----------	----------	----------
Experiment 1[3]															
n															
Diet	PS-2	49.24^{b}	50.46	50.49	52.95^{a}	52.52^{b}	53.81	55.41^{b}	57.49	56.28	55.83^{b}	57.70	59.50	59.17	55.34^{b}
	PS	49.28^{b}	51.09	52.36	52.71^{a}	54.37^{b}	54.74	56.25^{b}	56.56	56.85	57.99^{ab}	57.53	58.90	61.47	56.08^{b}
	PS+2	50.92^{b}	49.50	50.51	50.05^{b}	53.71^{b}	54.34	57.89^{ab}	57.12	56.75	57.41^{ab}	57.73	59.92	59.89	55.97^{b}
	PS+4	53.46^{a}	51.89	53.24	53.61^{a}	57.41^{a}	54.37	56.04^{b}	57.11	58.03	58.63^{a}	59.37	60.19	60.76	57.26^{a}
	PS+6	51.40^{b}	53.16	50.61	52.69^{a}	55.01^{ab}	54.40	58.90^{a}	57.77	58.51	58.93^{a}	58.69	59.72	60.13	57.00^{a}
Overall mean		50.86	51.22	51.44	52.40	54.60	54.33	56.90	57.21	57.28	57.76	58.20	59.65	60.28	56.30
SEM		0.93	0.64	0.63	0.56	0.51	0.53	0.52	0.55	0.53	0.55	0.55	0.57	0.55	0.32
P value		*	NS	NS	*	*	NS	**	NS	NS	*	NS	NS	NS	**
Experiment 2															
n															
Diet	PS-2	46.79	48.90	48.72	50.66^{b}	53.43	53.61	52.28^{c}	54.54	54.82	52.54^{b}	54.74	54.99^{b}	56.10	52.79^{c}
	PS	49.09	50.23	50.12	55.54^{a}	55.09	52.31	55.23^{bc}	55.56	54.83	56.89^{a}	57.58	56.61^{ab}	57.51	54.73^{ab}
	PS+2	47.51	49.48	51.91	52.95^{ab}	53.34	52.85	55.88^{ab}	53.26	56.84	57.12^{a}	57.41	56.17^{b}	56.36	54.35^{ab}
	PS+4	49.47	49.58	49.92	53.29^{ab}	52.48	52.28	54.25^{bc}	53.20	55.48	57.14^{a}	55.75	56.60^{ab}	55.53	53.93^{b}
	PS+6	49.38	50.60	51.65	54.27^{a}	52.93	53.65	56.85^{a}	55.24	56.12	57.71^{a}	57.93	59.98^{a}	57.29	55.11^{a}
Overall mean		48.45	49.76	50.46	53.34	53.45	52.94	54.90	54.36	55.62	56.28	56.68	56.87	56.56	54.19
SEM		0.85	0.69	0.74	0.53	0.82	0.82	0.79	0.87	0.56	0.71	0.81	0.78	0.91	0.72
P value		NS	NS	NS	*	NS	NS	*	NS	NS	**	NS	**	NS	**

[a, b, c] Within and experiment, means within a column (dietary treatment) with no common superscript differ significantly.

[1] Treatment name. PS = Photostimulation (20 wk), the number refers to the number of wk before (-) or after (+) photostimulation that the birds were switched from a low Ca grower diet to a high Ca breeder diet.

[2] Egg weight over the course of the experiment.
[3] In Experiment 1, birds were maintained at 18 C throughout the study, in Experiment 2, the birds were housed at 28 C throughout the trial, n = 25 observations per treatment; each observation was the average of one egg per hen per sampling day. If a hen did not lay on the sampling day, it was excluded from that day's analysis.
* < 0.05, ** < 0.01.

Table 2-4. Mean shell weight of Ross 508 hens housed at 18C (Experiment 1) or 28C (Experiment 2) and fed one of five dietary treatments

		Hen Age (wks)													
Main Effect	Trt[1]	25	25.4	26	26.4	27	27.4	28	28.4	29	29.4	30	30.4	31	Overall[2]
		(g)													
Experiment 1[3]															
n															
Diet	PS-2	4.12	4.68	4.70^{ab}	4.82	5.06^{b}	5.02^{ab}	5.13	4.73	4.71^{ab}	4.99	5.34	5.40	5.30	4.99^{b}
	PS	4.56	4.78	4.97^{a}	4.85	5.18^{ab}	4.82^{b}	5.13	4.68	4.62^{b}	5.15	5.14	5.13	5.34	4.98^{b}
	PS+2	4.53	4.68	4.60^{ab}	4.58	5.12^{ab}	5.03^{ab}	5.35	4.79	4.63^{b}	5.14	5.20	5.35	5.49	5.02^{ab}
	PS+4	4.61	4.56	5.01^{a}	4.86	5.44^{a}	5.22^{a}	5.22	4.71	4.94^{a}	5.07	5.38	5.33	5.30	5.10^{a}
	PS+6	4.25	4.64	4.27^{b}	4.52	4.94^{b}	4.98^{ab}	5.25	4.57	4.78^{ab}	5.05	5.28	5.23	5.31	4.95^{b}
Overall mean		4.41	4.67	4.71	4.73	5.15	5.01	5.22	4.70	4.74	5.08	5.27	5.29	5.35	5.01
SEM		0.09	0.08	0.07	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.57
P value		NS	NS	*	NS	*	*	NS	NS	*	NS	NS	NS	NS	*
Experiment 2															
n															
Diet	PS-2	4.12	4.40^{abc}	4.67^{ab}	4.69^{b}	5.02^{ab}	4.89	4.84^{b}	4.24	4.41	4.59^{b}	4.87^{b}	4.91^{b}	4.93^{ab}	4.67^{b}
	PS	4.31	4.65^{ab}	4.79^{a}	5.07^{a}	5.14^{a}	4.81	5.11^{ab}	4.50	4.41	4.95^{a}	5.10^{ab}	5.10^{ab}	5.18^{a}	4.89^{a}
	PS+2	4.64	4.76^{a}	4.88^{a}	4.92^{a}	5.04^{a}	4.86	5.22^{a}	4.38	4.63	5.04^{a}	5.23^{a}	4.94^{b}	4.89^{ab}	4.90^{a}
	PS+4	4.22	4.30^{bc}	4.64^{ab}	4.82^{ab}	5.10^{a}	4.78	4.95^{ab}	4.33	4.45	5.04^{a}	4.91^{ab}	4.86^{b}	4.77^{b}	4.75^{b}
	PS+6	4.12	4.13^{c}	4.31^{b}	4.47^{b}	4.70^{b}	4.89	5.06^{ab}	4.53	4.58	5.28^{a}	5.17^{ab}	5.35^{a}	5.08^{ab}	4.77^{b}
Overall mean		4.28	4.45	4.66	4.79	5.00	4.85	5.04	4.40	4.50	4.98	5.06	5.03	4.97	4.80
SEM		0.10	0.07	0.07	0.06	0.05	0.06	0.05	0.06	0.06	0.06	0.06	0.06	0.06	0.59
P value		NS	*	*	*	*	NS	*	NS	NS	*	*	*	*	**

[a, b, c] Within an experiment. means within a column (dietary treatment) with no common superscript differ significantly.

[1] Treatment name. PS = Photostimulation (20 wk), the number refers to the number of wk before (-) or after (+) photostimulation that the birds were switched from a low Ca grower diet to a high Ca breeder diet.

[2] Shell weight over the course of the experiment.
[3] In Experiment 1, birds were maintained at 18 C throughout the study, in Experiment 2, the birds were housed at 28 C throughout the trial.n = 25 observations per treatment; each observation was the average of one egg per hen per sampling day. If a hen did not lay on the sampling day, it was excluded from that day's analysis.
* < 0.05, ** < 0.01.

Table 2-5. Mean shell weight as a percentage of Ross 508 hens housed at 18C (Experiment 1) or 28C (Experiment 2) and fed one of five dietary treatments

		Hen Age (wks)													
Main Effect	Trt[1]	25	25.4	26	26.4	27	27.4	28	28.4	29	29.4	30	30.4	31	Overall[2]
		----------	----------	----------	----------	----------	----------	(%)	----------	----------	----------	----------	----------	----------	----------
Experiment 1[3]															
n															
Diet	PS-2	8.37^{b}	9.28	9.32^{a}	9.11^{ab}	9.64^{a}	9.35^{ab}	9.26	8.25^{b}	8.36	8.93	9.25	9.08	8.96	9.02^{a}
	PS	9.25^{a}	9.37	9.49^{a}	9.20^{a}	9.50^{a}	8.81^{cd}	9.13	8.28^{b}	8.13	8.88	8.93	8.71	8.73	8.88^{a}
	PS+2	9.03^{ab}	9.45	9.10^{ab}	9.14^{ab}	9.53^{a}	9.25^{abc}	9.27	8.42^{a}	8.16	8.95	9.00	9.08	9.17	8.99^{a}
	PS+4	8.68^{ab}	8.79	9.43^{a}	9.10^{ab}	9.49^{a}	9.65^{a}	9.34	8.23^{ab}	8.54	8.67	9.07	8.87	8.74	8.94^{a}
	PS+6	8.30^{b}	8.75	8.53^{b}	8.62^{b}	8.98^{b}	9.17^{bc}	8.97	7.92^{b}	8.18	8.61	9.02	8.77	8.84	8.69^{b}
Overall mean		8.73	9.13	9.17	9.03	9.43	9.25	9.19	8.22	8.27	8.81	9.05	8.90	8.89	8.91
SEM		0.14	0.12	0.10	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.84
P value		*	NS	**	*	*	*	NS	*	NS	NS	NS	NS	NS	**
Experiment 2															
n															
Diet	PS-2	8.81^{b}	8.99^{bc}	9.59^{a}	9.29^{a}	9.44^{a}	9.12	9.26	7.78	8.11	8.90	8.91	8.97	8.80	8.93^{ab}
	PS	8.86^{b}	9.27^{ab}	9.55^{a}	9.18^{a}	9.40^{a}	9.18	9.29	8.10	8.01	8.69	8.89	8.99	9.00	8.95^{a}
	PS+2	9.74^{a}	9.65^{a}	9.43^{a}	9.28^{a}	9.47^{a}	9.22	9.39	8.23	8.15	8.84	9.11	8.79	8.67	9.03^{a}
	PS+4	8.58^{b}	8.69^{cd}	9.33^{a}	9.08^{a}	9.75^{a}	9.13	9.13	8.13	8.02	8.83	8.81	8.61	8.59	8.82^{bc}
	PS+6	8.34^{b}	8.17^{d}	8.35^{b}	8.23^{b}	8.89^{b}	9.14	8.92	8.20	8.16	9.16	8.93	8.99	8.87	8.67^{c}
Overall mean		8.87	8.95	9.25	9.01	9.39	9.16	9.20	8.09	8.09	8.88	8.93	8.87	8.79	8.88
SEM		0.09	0.14	0.12	0.11	0.11	0.11	0.09	0.10	0.10	0.12	0.11	0.12	0.11	0.90
P value		*	**	**	**	*	NS	NS	NS	NS	NS	NS	NS	NS	**

[a, b, c, d] Within and experiment, means within a column (dietary treatment) with no common superscript differ significantly.

[1] Treatment name. PS = Photostimulation (20 wk), the number referes to the number of wk before (-) or after (+) photostimulation that the birds were switched from a low Ca grower diet to a high Ca breeder diet.

[2] Shell weight as a percentage of egg weight over the course of the experiment (measured on 3500 eggs; two per hen per week).
[3] In Experiment 1, birds were maintained at 18 C throughout the study, in Experiment 2, the birds were housed at 28 C throughout the trial,.n = 25 observations per treatment; each observation was the average of one egg per hen per sampling day. If a hen did not lay on the sampling day, it was excluded from that day's analysis.
* < 0.05, ** < 0.01.

Table 2-6. Tibiotarsus characteristics of 31 wk old Ross 508 hens housed at 18C (Experiment 1) or 28C (Experiment 2) and fed one of five dietary treatments

		Parameter Measured[1]					
Main Effect	Trt.[2]	Bone wt.	Ash wt.	Ca wt.	Ash/Bone	Ca/Bone	Ca/Ash
		(g)			(%)		
Experiment 1[3]							
n							
Diet	PS-2	4.66	2.29	0.79	49.50	18.15	36.67^{ab}
	PS	4.59	2.20	0.83	47.77	17.12	35.75^{b}
	PS+2	4.54	2.32	0.85	51.27	18.47	36.05^{b}
	PS+4	4.76	2.36	0.85	49.86	18.49	37.12^{a}
	PS+6	4.59	2.19	0.81	48.07	17.24	35.89^{b}
SEM		0.53	0.29	0.01	5.52	2.11	1.48
P value		NS	NS	NS	NS	NS	*
Experiment 2							
n							
Diet	PS-2	4.47	2.32	0.80	49.37	18.05^{a}	36.53^{a}
	PS	4.68	2.22	0.84	47.45	17.48^{ab}	36.86^{a}
	PS+2	4.62	2.19	0.81	47.42	16.51^{b}	34.76^{b}
	PS+4	4.70	2.29	0.81	48.89	18.29^{a}	37.43^{a}
	PS+6	4.95	2.22	0.82	45.18	16.48^{b}	36.48^{a}
SEM		0.52	0.26	0.02	4.46	1.82	1.70
P value		NS	NS	NS	NS	**	**

[a, b, c, d] Within an experiment, means within a column (dietary treatment) with no common superscript differ significantly

[1] Bone = dry bone weight, Ash = ash weight, Ca = g of Ca present in the bone, Ash/Bone = ash weight as a percentage of dry bone weight, Ca/Bone = g of Ca as a percentage of dry bone weight, Ca/Ash = g of Ca as a percentage of ash weight.

[2] Treatment name. PS = Photostimulation (20 wk), the number refers to the number of wk before (-) or after (+) photostimulation that the birds were switched from a low Ca grower det to a high Ca breeder diet.
[3] In Experiment 1, birds were maintained at 18 C throughout the study, in Experiment 2, the birds were housed at 28 C throughout the trial n = 25 observations per treatment.
[4] Hens were photostimulated at 20 wk of age. Dietary treatments refer to the number of wk relative to photostimulation that hens were switched from a 0.88% grower diet to a 2.96% Ca breeder diet. Hens were switched at 18, 20, 22, 24, and 26 wk of age (treatments PS-2, PS, PS+2, PS+4, and PS+6 respectively).
* < 0.05, ** < 0.01.

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3.0 THE EFFECT OF MIDNIGHT FEEDING ON FEED CONSUMPTION AND EGGSHELL QUALITY IN LAYING HENS[1]

3.1 ABSTRACT

The effect of allowing laying hens access to feed for 1 hr at midnight on eggshell quality and calcium status was investigated. Two 4.5-wk-long trials were performed using individually-caged, 60 wk old SCWL Shaver 2000 hens (48 hens/experiment). In Experiment 1, the hens were exposed to midnight feeding for 27 wk prior to the study, beginning at 33 wk of age. At 60 wk of age, half of the hens (n=24) were allowed continued midnight feeding (MF treatment) while the other half (n=24) had feed removed at 2000 h and replaced at 0500 h to prevent overnight feed intake (CONT treatment). In Experiment 2, the experimental conditions and treatments were as described in Experiment 1, except the hens had no prior exposure to midnight feeding. In each experiment, diurnal (0500 h to 2000 h), nocturnal (2000 h to 0500 h) and total daily feed consumption were recorded daily for each hen; Individual BW was recorded every two weeks. All eggs were collected and shell quality assessed. Egg weight, specific gravity, yolk weight, and dry shell weight were measured every two weeks, starting at 60 wk. The MF hens in Experiment 1 gained more weight during the experiment than CONT birds (MF, 49.3 g $\pm$ 94.4; CONT, -15.5 g $\pm$ 94.4; P = 0.0246) and BW gain relative to weight at the start of the experiment was also higher (MF, 2.52 % $\pm$ 5.07; CONT, -0.77 % $\pm$ 5.07; P = 0.0331); no differences in BW or BW change were observed in Experiment 2. Egg production did not differ in either study, although differences in total (MF, 27 $\pm$ 6; CONT, 24 $\pm$ 6; P = 0.0573) and marketable (MF, 27 $\pm$ 6; CONT, 23$\pm$ 6; P = 0.0600) egg production rates approached significance in Experiment 1. Egg weight, shell weight,

[1] Data from Experiment 1 in this chapter was generated by Carmen Zayac and Graham Olson as part of an ANSC 471 project.

specific gravity, and yolk weight were not different in either experiment. In Experiment 1 (prior exposure to midnight feeding), there were no differences between treatments in daytime or total weekly feed consumption. In Experiment 2 (no prior exposure to midnight feeding), daytime consumption of the CONT hens was higher for each week of the study. Under the conditions of the present studies, the midnight feeding program did not improve shell quality nor egg production.

3.2 INTRODUCTION

Laying hens have a large requirement for Ca in order to support normal metabolic processes, and especially eggshell formation. The ultimate source of this Ca is the diet. However, during periods of peak eggshell formation, hens cannot absorb Ca from the intestine rapidly enough to meet the requirements of the shell gland; during these periods, Ca is normally mobilized from the stores in the medullary bone, which can be rapidly depleted and replenished (Taylor, 1970).

The lighting program in a layer barn can affect feed consumption. In commercial laying operations, the lights are commonly off for 8 hours per day. Little, if any, feed consumption occurs during the dark period (Squibb and Collier, 1979). As a result, hens in commercial laying operations are limited as to when they can consume feed and therefore Ca. However, if hens are able to selectively consume particulate Ca (eg. oyster shell), they will consume little throughout the day and will preferentially consume the Ca at the end of the photoperiod (Mongin and Sauveur, 1974). Increasing Ca consumption at the end of the photoperiod ensures that the hen has Ca readily available in the digestive tract during the dark period when demand for Ca is typically at its peak for eggshell formation (Etches, 1987).

The feed consumption of laying hens under diurnal feeding programs has fallen due to intensive breeding for increased egg production (Roland and Bryant, 1994). This may further aggravate the problems of inadequate Ca available in the digestive tract during the nocturnal period.

Turning the lights on in the middle of the dark period (midnight feeding), is expected to stimulate the hens to consume feed resulting in Ca in the digestive tract during the night. By providing the hen with dietary Ca during the dark period, midnight feeding may reduce the dependence and utilization of medullary bone

reserves and improve eggshell quality. Work in our laboratory has shown that midnight feeding can be an effective means of reversing the deleterious effects of a metabolic Ca insufficiency (unpublished observations). Previous research by Harms *et al.* (1996) involved turning the lights in the barn on, and running the feeders for 45 min every night. After 4 days of midnight feeding, eggshell quality was measured. The results indicated that there was an increase in the specific gravity, shell weight, and percent shell in the eggs from the midnight fed hens.

The objective of the current experiment was to study the effects of long-term and short-term midnight feeding on feed consumption, eggshell quality, and egg production of laying hens and the effect of long-term midnight feeding on bone mineral density, Ca content, and breaking strength. The hypothesis tested was that midnight feeding (both short- and long-term) would increase feed consumption, egg production, eggshell quality, and bone mineral content.

3.3 MATERIALS AND METHODS

Stocks and Housing

The study consisted of two experiments, each using 48 Single Comb White Leghorn Shaver 2000 hens. The hens were individually caged and fed a commercial-type laying hen mash (Table 3-1). The hens were 60 wk of age at the start of the experiments and 64.5 wk of age when the experiments ended.

Experimental Design

The hens used in Experiment 1 were part of a research flock in which a decrease in egg production, eggshell quality and bird skeletal health were observed. A midnight feeding program was instituted at 33 wk of age, and an almost immediate improvement in egg production, eggshell quality and bird health were observed (unpublished data). Therefore, all hens (n = 48) in Experiment 1 were exposed to

light and *ad libitum* feed from 0500 h to 2000 h (15L:8D) and from 2400 h to 0100 h prior to data collection beginning at 60 wk of age. The lights were only turned on for 1 h so that the birds would not perceive the light as the start of a new day. Experiment 2 was conducted in the same facility, using the same strain of hens, one year later. Experimental conditions and treatments were the same for Experiment 2 as for Experiment 1, except the hens had no exposure to midnight feeding prior to 60 wk of age.

In each experiment, 48 hens were divided into two treatment groups labelled control (CONT, n = 24) and midnight fed (MF, n = 24). Feed consumption of the individually- caged and fed hens was measured and recorded daily. A known amount of feed (greater than expected feed consumption; no hen ever consumed all of the feed in the feeder) was allocated to each hen immediately before the lights in the barn came on at 0500 h. After the lights went off at 2000 h, the feeders were removed and weighed. The feed consumed during this period was defined as the diurnal feed consumption. The feeders of the CONT treatment remained off from 2000 h to 0500 h the next day, while those of the MF treatment were immediately replaced. When the lights went on at 2400 h, all hens were exposed to light, however only those in the MF treatment had access to feed. The feeders were weighed again just before 0500 h and the feed consumed during this time was defined as nocturnal feed consumption. Thus, total feed consumption for the CONT treatment occurred between 0500 h and 2000 h (diurnal feed consumption) whereas total feed consumption for the MF treatment resulted from adding the diurnal feed consumption to the nocturnal feed consumption.

Eggs were collected at 1300 h each day over the course of the experiment; each egg was identified with the hen and date of production. Each egg was weighed

and a grade was assigned to each egg to describe the condition of the egg or eggshell (normal, soft shelled, no shell, double yolk, broken, abnormal or pecked). At 60, 62, and 64 wk of age, eggs were collected and stored overnight. Specific gravity was measured the next day using the flotation method (Nordstrom and Ousterhout, 1982) in saline solutions ranging from 1.062 to 1.094 with the saline concentration increasing by 0.002 in each subsequent container. After floating, the eggs were dried, broken, and yolk and dry eggshell weights were obtained. From this, shell weight as a percentage of egg weight was calculated. Individual hen body weights were measured at 60, 62, and 64 wk of age; weight gain as a percentage of 60 wk body weight was calculated. At 64.5 wk of age, the hens were killed by cervical dislocation.

Bone Mineral Analysis

After the birds were killed, the left leg was removed from the carcass and frozen for further analysis. Once the leg was thawed and soft tissue and toes removed, tibia cross-sectional area and density were measured at the mid-point using a Norland XCT[2] scanner with XMENU software version 5.40C (Korver et al., 2003). An inner threshold level was set at 400 mg/cm^3 to separate cortical and trabecular bone, and it was assumed that trabecular bone reflected the amount of medullary bone present. The tibia were dried for 24 h (100 C) and dry bone weight determined. The dried bones were placed in an ashing oven (600 C) for 16 h and the ash dissolved in a hydrochloric acid solution. The Ca content of the solution was measured using a Perkin-Elmer Model 4000 Atomic Absorption Spectrophotometer[3] and compared to standard solutions of known Ca content. In Experiment 1 only, the breaking strength of the bones was measured prior to drying and ashing using an Instron Materials

[2] Norland Corp., Fort Atkinson, WI, USA 53538
[3] Perkin Elmer, Norwalk CT, USA 06856

Tester[4] with Automated Materials Test System software version 8.09, a standard 50 kg load cell, and a modified sheer plate (8 cm in length and 1 mm in width). A 3 cm distance between the two fixed points supporting the bone, and a crosshead speed of 100 mm/minute were held constant throughout each measurement.

Statistical Analysis

The main effect in both experiments was the feeding program; the experimental unit was the individual bird. Data were analysed as a one-way ANOVA using the General Linear Models (GLM) procedures of SAS® (SAS Institute, 1999). Least square means were compared using the pdiff procedures of SAS. All statements of significance were assessed at $P \leq 0.05$. Two hens in Experiment 1, one from each treatment died during the course of the experiment and were removed from the data set. The causes of death were unrelated to the experimental treatments or Ca metabolism.

3.4 RESULTS AND DISCUSSION

Body Weight and Feed Consumption

Experiment 1. In the long-term midnight feeding study, there were no differences in body weight between the CONT and MF hens at any time point. However, over the course of the experiment (from 60 to 64 wk of age), the MF hens gained more weight than the CONT hens ($P = 0.0246$; Table 3-2). Weight gain as a percentage of 60 wk body weight was also greater in the MF hens ($P = 0.0331$). There were no differences in daytime feed consumption between the MF and CONT treatments. At wk 1 and 4 ($P = 0.0663, 0.0608$ respectively; Table 3-3), the MF hens consumed a nearly significantly greater amount of feed. Hurwitz and Bar (1969) reported that when given free access to Ca (e.g. oystershell), hens would preferentially

[4] Model 4411, Instron Corp., Canton, MA, USA 02021

consume more Ca on days they were forming an eggshell than on non-shell-forming days. Feed consumption of hens on days when eggshell formation is occurring is increased by 25 % over days when no shell is being formed (Johnson, 1986). Midnight feeding gives hens the opportunity to consume feed when it is required and may explain the propensity of the MF hens to consume more total feed than their CONT counterparts. This indicates that having access to feed, and especially feed with appropriate levels of Ca during eggshell formation is important and will affect eggshell quality. It is possible that the CONT hens, which had previously adjusted to consuming feed in the overnight period, lost weight as a result of the change in feeding pattern. Although the differences in overall feed consumption only approached significance, they may have been enough to cause the differences in weight described above; a significant weight loss or gain is not necessarily the result of a significant decrease or increase in feed consumption. Squibb and Collier (1979) determined that broilers, when given alternating 12 h periods of light and dark increased feed consumption 2 to 3 h before the lights went out as compared to birds in all-light or all-dark treatments. In the same manner, the CONT hens may have increased their diurnal consumption in anticipation of the nocturnal period without feed. Given that broilers are more motivated to eat than Leghorns (Squibb and Collier, 1979), the hens in the present experiment probably consumed the feed during the 1 hour of light, rather than when the lights were out. Harms *et al.* (1996) reported that previous attempts at midnight feeding involving hand-feeding, such as performed in the present study, were not successful as the hens would not consume feed when the lights were turned on at midnight. However, when the experiment was performed using automatic feeders, the hens responded to the sound cue by consuming feed. In the current experiment, the hens were accustomed to hand feeding before the trial

started, so it is possible a similar sound cue would not have encouraged significantly greater consumption. Had the experiment continued, the differences in body weight gain would probably be eliminated as the CONT hens adjusted to the midnight feeding schedule.

Experiment 2. In the short-term midnight feeding study, there were no differences between the CONT and MF hens in body weight or gain at any week (Table 3-2). Total feed consumption was the same for both treatments throughout the experiment, however, the CONT treatment had a higher average daytime feed consumption than the MF treatment for each week (Table 3-3). As a major proportion of eggshell formation occurs at night (Etches, 1987), it is possible that the MF hens in this short-term study responded by increasing nocturnal feed intake at the expense of diurnal intake, without changing total daily consumption. Thus, hens given access to feed during active eggshell formation (e.g. the MF treatment) may be more motivated to consume fed than during times when a shell is not being formed. Differences in intake patterns between hens exposed to short-term (Experiment 2) and long-term (Experiment 1) midnight feeding are puzzling, and may be due to the relative novelty of the feeding program.

Previous research has shown that hens given the choice between Ca-deficient and Ca-rich diets will preferentially consume the Ca-rich diet when eggshell formation is occurring (Hurwitz and Bar, 1966). The current study could not confirm those results, as feed consumption was measured only twice per day. However there was no significant difference in total feed consumption between the two treatments. A possible explanation may lie in the Ca status of the hens before the experiments started. Hurwitz and Bar (1965) found that when dietary Ca levels were 3.56 % or higher, Ca from the intestine was the primary source of eggshell Ca. At dietary Ca

levels lower than 1.95 %, the medullary bone reserves provided 30 to 40 % of the Ca required for eggshell formation. The other 60 to 70 % Ca required for eggshell formation comes directly from the diet. If hens have insufficient dietary Ca and medullary bone reserves, poor eggshell quality and metabolic disorders can result (Couch, 1955). The hens in the current experiment were fed adequate Ca throughout the grower and pre-lay periods and, although not measured, should have been in a positive Ca balance at the beginning of the experiments. Perhaps, if the hens were in a negative Ca balance, the MF treatment would have been more motivated to consume feed.

Egg Production and Egg Traits

Experiment 1. The MF hens produced 27 eggs, of which all 27 were marketable over the 32 days of the experiment. The CONT hens produced 24 eggs of which 23 were marketable. The difference between total and treatments in marketable eggs approached significance (P = 0.0573 and 0.0600 respectively). The weight loss exhibited by the CONT hens and the additional feed consumption of the MF hens, while not significant, may be associated with the difference in egg production. There were no differences between the two treatments in any of the egg traits measured (Table 3-4).

Experiment 2. No differences existed in the number of total or marketable eggs produced between treatments over the 32 days of the experiment. As in Experiment 1, there were no differences between the treatments in any of the egg traits measured (Table 3-4).

Supplying Ca to the gut when eggshell formation is occurring to increase eggshell quality has been well documented. Roland *et al.* (1973) found that the specific gravity of eggs laid in the afternoon is higher than that of eggs laid in the

morning. This was due to the availability of Ca directly from feed for a greater proportion of the eggshell calcification process rather than from medullary bone sources. This effect was not observed in the current experiment. Based on nocturnal feed consumption, the MF hens would be expected to have had feed in the gut during the day and for a proportion of the night.

It is interesting to note that the MF hens in Experiment 1 produced a nearly significantly greater number of both total and marketable eggs, and also had a nearly significant increase in feed consumption at several time points. This, coupled with the lack of effect in eggshell characteristics suggests that the hens in Experiment 1 were not limited by diet or calcium reserves to produce eggs. The lack of effects of the midnight feeding program on total feed intake, egg production and shell quality suggest a similar status in Experiment 2.

Bone Mineral Analysis

There were no differences in bone weight, ash weight, Ca, ash weight as a percentage of bone weight, Ca as a percentage of ash weight, or Ca as a percentage of bone weight (Table 3-5). Bone density was not affected by treatment. Cheng and Coon (1990) determined dry weight and ash weight of the whole bone was an indicator of daily Ca intake and could therefore be used to reflect bone status.

It was hypothesized that the MF treatment would reduce the dependence on medullary bone for eggshell formation by supplying feed to the gut for absorption during the day and especially overnight when a large proportion of eggshell formation takes place (Simkiss, 1967). Eggshell quality and bone quality were the same between the CONT and MF treatment groups in both experiments, suggesting that the hens were never over-dependant on bone stores of Ca for egg production. It appeared

that the hens in both experiments were able to mobilize sufficient Ca from the diet to support both eggshell formation and the maintenance of bone Ca reserves.

A wide variety of production and compositional analyses were performed in this study; it is clear that the midnight feeding program employed did not affect production or skeletal health. The present data contradicted previous observations by our group and work by others regarding the efficacy of midnight feeding programs. Based on production traits, shell quality, and bone mineralization, it was clear that the hens in both treatments were not impaired in their ability to absorb and metabolize Ca from the diet. Under conditions where Ca status may be impaired, such as dietary deficiency, a loss of appetite due to high environmental temperature, improper pre-lay nutrition, or impaired ability to form medullary bone, midnight feeding may provide additional metabolizable Ca and prevent Ca-related problems.

Table 3-1. Ingredient and calculated nutrient composition of laying hen diet

Ingredient	Amount of Ingredient
	----------g/Kg ----------
Wheat, Hard Red Spring	633.91
Soybean Meal, 48% CP	199.0
Corn Gluten Meal	20.0
Canola Oil	27.4
Limestone	95.2
Dicalcium Phosphate	10.75
Choline Cl Premix[1]	5.0
Layer Premix[2]	5.0
NaCl	2.5
D,L-methionine[3]	1.24
Nutrient composition	
Crude Protein, % (analyzed)	20.4
ME, kcal/kg	2,833
Calcium, % (analyzed)	3.89
Available Phosphorous, %	0.35
Lysine, %	0.83
Methionine, %	0.41
Methionine + Cystine, %	0.74
Linoleic acid, %	1.49

[1]Choline chloride premix provided 100 mg per Kg of diet. BASF Toronto, ON. N1G 4T2.
[2]Layer premix provided the following (per kg of diet): Vitamin A, 10,000 IU; vitamin D_3, 2,500 IU; vitamin E, 35 IU, vitamin K, 2.0 mg; pantothenic acid, 14.0 mg; riboflavin, 5.0 mg; folacin, 0.8 mg; niacin, 65.0 mg; thiamine, 2.0 mg; pyridoxine, 4.0 mg; vitamin B_{12}, 0.015 mg; biotin, 0.18 mg; iodine, 0.5 mg; manganese, 70.0 mg; copper, 8.5 mg; zinc, 80.0 mg; selenium, 0.1 mg; iron, 100.0 mg. BASF Toronto, ON. N1G 4T2.
[3]Degussa AG Canada Inc. Burlington ON. L7R 3Y6.

Table 3-2. Effect of midnight feeding on mean body weight and weight gain of laying hens

	Experiment 1[1]				Experiment 2[2]			
	Treatment[3]				Treatment			
Age (wk)	MF	CONT	*P* Value	Pooled SEM	MF	CONT	*P* Value	Pooled SEM
	---------Kg---------				-------- Kg--------			
60	1.812	1.814	NS	0.249	1.835	1.808	NS	0.180
62	1.807	1.779	NS	0.257	1.830	1.794	NS	0.184
64	1.854	1.804	NS	0.281	1.834	1.804	NS	0.198
	----------g----------				----------g----------			
Gain[4]	49.3	-15.5	0.0246	94.4	-1.1	-3.42	NS	67.2
	----------%---------				----------%---------			
% Gain[5]	2.52	-0.77	0.0331	5.07	-0.12	-0.20	NS	3.89

[1] Midnight feeding treatment applied from 33 to 64.5 weeks of age, data was collected from 60-64.5 wk of age by Carmen Zayac and Graham Olsen as part of an AnSc 471 project.

[2] Midnight feeding treatment applied from 60 to 64.5 weeks of age.

[3] MF = midnight feeding treatment (lights on and access to feed from 0500 h to 2000 h and from 2400 h to 0100 h each day, n = 24), CONT = control treatment (lights on and access to feed from 0500 h to 2000 h, lights on without access to feed from 2400 h to 0100 h, n = 24).

[4] Weight gain over the course of the experiment.

[5] Percent gain (weight gain as a percent of 60 wk body weight).

Table 3-3. Effect of midnight feeding on average daily feed consumption of laying hens

Age	60			61			62			63			64		
Treatment[3]	Diurnal[4]	Nocturnal[5]	Total[6]	Diurnal	Nocturnal	Total	Diurnal	Nocturnal	Total	Diurnal	Nocturnal	Total	Diurnal	Nocturnal	Total
	Experiment 1[1]														
	g														
MF	107	13	120	108	14	122	105	17	122	112	18	130	113	13	126
CONT	100	----	100	112	----	112	106	----	106	111	----	111	110	----	110
Pooled SEM	34	----	36	35	----	38	36	----	38	32	----	34	31	----	33
P Value	NS	----	NS	NS	----	NS	NS	----	NS	NS	----	NS	NS	----	0.1005
	Experiment 2[2]														
	g														
MF	105	12	117	108	14	122	109	15	124	108	15	123	111	17	128
CONT	120	----	120	123	----	123	124	----	124	124	----	124	124	----	124
Pooled SEM	19	----	20	17	----	17	18	----	18	15	----	15	17	----	17
P Value	0.0071	----	NS	0.0028	----	NS	0.0074	----	NS	0.0008	----	NS	0.0109	----	NS

[1] Midnight feeding treatment applied from 33 to 64.5 weeks of age, data was collected from 60-64.5 wk of age by Carmen Zayac and Graham Olsen as part of an AnSc 471 project.
[2] Midnight feeding treatment applied from 60 to 64.5 weeks of age.
[3] MF = midnight feeding treatment (lights on and access to feed from 0500 h to 2000 h and from 2400 h to 0100 h each day), CONT = control treatment (lights on and access to feed from 0500 h to 2000 h, lights on without access to feed from 2400 h to 0100 h).
[4] Average diurnal feed consumption (feed consumed from 0500 h to 2000 h).
[5] Average nocturnal feed consumption (feed consumed from 2400 h to 0100 h).
[6] Average total daily feed consumption.

Table 3-4. Effect of midnight feeding on egg traits from laying hens

	Experiment 1[1]				Experiment 2[2]			
	Treatment[3]				Treatment			
	MF	CONT	*P* value	Pooled SEM	MF	CONT	*P* value	Pooled SEM
Egg Weight (g)	65.7	64.2	NS	2.4	64.7	63.9	NS	4.0
Shell Weight (g)	5.6	5.6	NS	0.5	5.5	5.4	NS	0.3
Percent Shell (%)[4]	8.5	8.7	NS	0.8	8.6	8.5	NS	0.5
Specific Gravity	1.077	1.078	NS	0.003	1.078	1.077	NS	0.004
Yolk Weight (g)	18.5	18.0	NS	0.8	17.7	18.0	NS	0.8

[1] Midnight feeding treatment applied from 33 to 64.5 weeks of age, data was collected from 60-64.5 wk of age by Carmen Zayac and Graham Olsen as part of an AnSc 471 project.
[2] Midnight feeding treatment applied from 60 to 64.5 weeks of age.
[3] MF = midnight feeding treatment (lights on and access to feed from 0500 h to 2000 h and from 2400 h to 0100 h each day, n = 24), CONT = control treatment (lights on and access to feed from 0500 h to 2000 h, lights on without access to feed from 2400 h to 0100 h, n = 24).
[4] Shell weight as a percentage of egg weight.

Table 3-5. Effect of midnight feeding on bone characteristics from laying hens

	Experiment 1[1]				Experiment 2[2]			
	Treatment[3]				Treatment			
Parameter Measured[4]	MF	CONT	*P* value	Pooled SEM	MF	CONT	*P* value	Pooled SEM
Bone (g)	4.34	4.30	NS	0.54	3.85	3.87	NS	0.66
Ash (g)	2.69	2.73	NS	0.44	2.14	2.15	NS	0.59
Ca (g)	0.87	0.88	NS	0.15	0.75	0.70	NS	0.01
Ash/Bone (%)	62.0	62.8	NS	6.70	49.9	48.9	NS	8.91
Ca/Bone (%)	20.1	20.3	NS	2.46	17.7	17.6	NS	1.77
Ca/Ash (%)	32.4	32.3	NS	1.37	31.6	32.3	NS	19.73
Total Density (mg/cm^3)	634	611	NS	69	627	616	NS	72
Total Area (mm^2)	32	33	NS	2	29	23	NS	2
Trabecular Density (mg/cm^3)	202	206	NS	35	198	197	NS	29
Trabecular Area (mm^2)	13	14	NS	3	13	12	NS	4
Cortical Density (mg/cm^3)	995	1009	NS	52	967	959	NS	63
Cortical Area (mm^2)	17	16	NS	3	15	14	NS	2

[1] Midnight feeding treatment applied from 33 to 64.5 weeks of age, data collected by Carmen Zayac and Graham Olsen as part of an AnSc 471 project.

[2] Midnight feeding treatment applied from 60 to 64.5 weeks of age.

[3] MF = midnight feeding treatment (lights on and access to feed from 0500 h to 2000 h and from 2400 h to 0100 h each day, n = 24), CONT = control treatment (lights on and access to feed from 0500 h to 2000 h, lights on without access to feed from 2400 h to 0100 h, n = 24).

[4] Bone = dry bone weight, Ash = ash weight, Ca = g of Ca present in the bone, Ash/Bone = ash weight as a percentage of dry bone weight, Ca/Bone = g of Ca as a percentage of dry bone weight, Ca/Ash = g of Ca as a percentage of ash weight.

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4.0 GENERAL DISCUSSION

Poor eggshell quality causes numerous problems in the broiler breeder and table egg industries every year. In the broiler breeder industry, where producers are paid on the basis of saleable chicks, poor eggshell quality results in reduced hatchability and a financial loss. In the table egg industry, eggs with poor shells cannot be sold as table eggs, and must instead be sold into the further processing market at a much lower price. Spent hens are no longer being accepted for use in traditional further processing as the leg and wing bones are too fragile. Instead, the hens are rendered for use in animal feed which is an added cost to the producer rather than getting compensated for the birds. Therefore, bone breakage as a result of mineral depletion is fast becoming important in the table egg industry both as an economical and an animal welfare issue. While the concerns are similar between the two industries, the solutions are not.

In broiler breeders, the pressure to produce broiler chicks with high growth potential has led to years of selection for growth rate and appetite in the male lines, and egg production in the female lines. Together, this results in breeders that cannot be given *ad libitum* access to feed or they will over-consume, leading to decreased fertility and leg problems. Feed restriction reduces Ca intake and limits the amount of time in which birds have access to feed. In a typical broiler breeder operation, the day's feed is given once, early in the morning. Feed is therefore not available directly from the digestive tract during the overnight period when most eggshell formation is occurring (Roland *et al.* 1973). To supply Ca for eggshell formation, hens have a specialized type of bone called medullary bone, that serves as a readily accessible reservoir for Ca (Simkiss, 1967). Medullary bone is broken down in times of Ca shortfall, and re-mineralized when

there is available Ca in the gut that is not immediately required for eggshell formation. Medullary bone is formed approximately 2 weeks before first egg and is accompanied by an increased physiological demand for Ca (Hurwitz and Bar, 1969).

4.1 Ca NUTRITION IN BROILER BREEDER HENS

In commercial broiler breeder operations, the birds are supplied with a grower diet (approximately 1 % Ca) or a pre-breeder diet (approximately 2 % Ca) until the flock reaches 5 % production. In order to meet the dietary needs of the majority of the flock, the feed is then changed to a high Ca breeder diet (over 3 % Ca). Several factors can influence the timing of this dietary change but one of the most important is flock uniformity in body weight. If a flock is not uniform in body weight and stage of sexual maturity, some birds can begin production weeks before others, creating a dilemma as to when to increase Ca; to meet the requirements of the first birds or the last to enter production. The research presented in this thesis indicated that increasing Ca too soon, such as 2 weeks before photostimulation (PS-2; Chapter 2), can have detrimental effects on eggshell quality and bird health. Conversely, supplying Ca after the hens begin production and have an increased need for Ca, such as 6 weeks after photostimulation (PS+6; Chapter 2), does not meet the nutritional needs of the flock. The results showed that, in a thermoneutral environment (Experiment 1; Chapter 2), there were no detrimental effects to increasing Ca before photostimulation. However, in a high-temperature environment (Experiment 2; Chapter 2), the timing of increasing Ca became more important. Peak egg production averaged across the dietary treatments in the thermoneutral environment was almost 20 % higher and the birds reached peak 2 weeks later than in the hot environment. In addition, eggshell quality in the PS-2 and PS+6

treatments was compromised when the hens were exposed to heat stress. Contradictory results were seen for bone characteristics. In the thermoneutral environment (Chapter 2), bone characteristics were the worst in the PS (diet changed at photostimulation), PS+2 (diet changed 2 wk after photostimulation), and PS+6 treatments. The PS+2 treatment also showed the worst bone characteristics in the high-temperature environment. The results suggest that eggshell quality was maintained at the expense of bone quality. The results discussed here are recommendations based on the experiment performed in which the birds were photostimulated at 20 wk. It is important to remember that the decision of when to increase dietary Ca must be made based on when medullary bone is expected to form in the flock, based on when the birds are photostimulated. Proper supervision and management on a flock-by-flock basis will better determine the best time to increase Ca rather than arbitrarily choosing an age at which Ca should be increased.

When reviewing the results, it is important to keep in mind the practical application to industry. In commercial broiler breeder operations, it is common for the pullets to be moved into a laying barn at approximately 22 wk of age, and vaccinated at the same time. The vaccination involves penning up the birds, picking each up by hand, and injecting the vaccine with a needle. In addition, soon after the hens are transferred, the males are added to the barn, adding further stress. Besides being moved, broiler breeder hens are subjected to the ongoing stress of feed restriction. While it is difficult to compare one type of stress with another, we can speculate that any of these procedures might exacerbate the effects of the stress felt by the hens in the high environmental temperature treatment and make the results relevant to a commercial operation.

In all experiments there are restrictions placed on the experimental design by cost, manpower, and time. The same is true in the current studies. The quantitative computed tomography (QCT) used in Chapter 3 was not available when running the experiment discussed in Chapter 2. The ability to see changes in bone density *in vivo* would have added valuable data. In addition, many metabolic disorders become apparent as hens age, so lengthening the experiment to include the entire production cycle may have given some insight to the effects of pre-lay nutrition in broiler breeders hens; a valuable resource since all of the research in the area has been performed on Leghorns. It is well documented that one of the most important predictors of bone quality is the housing in which birds are maintained (Fleming *et al.* 1994). Birds reared in cages typically show signs of osteoporosis and other metabolic disorders more often than those reared on the floor. Unlike commercial barns, the birds used in the current experiment were in cages to measure individual feed consumption, however, had the hens been kept on the floor, different results may have been observed.

4.2 CA NUTRITION IN LAYING HENS

The concerns in the table egg industry are two-fold; poor eggshell quality and poor bone strength. In order to increase bone strength, and consequently eggshell quality, midnight feeding has been suggested as a way of supplying Ca to the hens overnight when it can be directly deposited into the eggshell, rather than depleting medullary bone. In the Chapter 3, midnight feeding did not affect eggshell or bone quality in either experiment. However there were subtle differences in feed consumption between the two experiments. When the hens were subjected to long-term midnight feeding, the total feed consumption of the midnight fed (MF) hens was almost significantly different from that

of the daytime-fed (CONT) hens 60 and 63 wk of age, while the diurnal feed consumption was the same. When the hens were exposed to short-term midnight feeding, the diurnal feed consumption of the CONT hens was higher than that of the MF hens for every wk except 62 wk of age. Nonetheless, the total feed consumption was not different between the two groups. The results suggested that the additional feed available to the MF hens during the nocturnal period was not used to increase eggshell quality. Previous research has stated that if given free-choice access, hens will consume more feed when forming an eggshell. The results from Chapter 3 suggested that the Ca requirements of the hens were met by the amount of feed available to the CONT hens. The MF hens appeared to spread out their feed consumption over the diurnal and nocturnal periods but did not consume additional feed.

Although previous studies have been performed looking at midnight feeding, it is unclear what the Ca status of the hens in the current study was when midnight feeding began. While not measured, the experiments performed in Chapter 3 were performed on hens showing no outward signs of Ca deficiency. The results may have been different if the hens were in a negative Ca balance when the experiments began, even if it was induced by unusually low dietary Ca levels. The QCT technology was only available after the trials were complete, however, the bone density at the beginning of the experiments would have been a useful tool to determine if there was any change over time as well as between treatments. Observing changes in bone quality throughout the production cycle from an *in vivo* point of view would be invaluable. This research is important, not only from a production standpoint, but from an animal welfare standpoint

as well. As agriculture moves further into the future, animal welfare is becoming a larger issue in the poultry industry.

Both experiments performed in this field of study demonstrated that simply providing more Ca does not guarantee increased shell and bone quality. The timing of providing Ca to flocks in relation to medullary bone formation and the onset of egg production is critical to eggshell quality and maintaining marketable egg production. Stress also affects how effective the timing of a particular feeding program is, making the decision of when to increase dietary Ca much more critical. In addition, the time of day in which the hens have access to feed can influence their ability to produce and maintain high quality eggshell and prolific egg production. Although the current results showed no differences in eggshell or bone quality in midnight fed hens as compared to those on a diurnal feeding system, previous studies have demonstrated that midnight feeding is effective. The experiments were designed and conducted differently which may explain the discrepancies found in the results. However, both experiments exhibit that the timing of Ca intake plays an important role in maintaining or improving the Ca metabolism of egg laying birds.

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